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COMPARATIVE ANATOMY OF THE MORACEAE AND THEIR PRESUMED ALLIES[1]

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(WITH SIXTY-THREE FIGURES)

Introduction

It has become increasingly evident that if botanists are ever to establish a natural classification of the angiosperms, this natural system will be the combined work of taxonomists, anatomists, cytologists, paleobotanists, and specialists in related fields of research. Most of the present day systems are based on the morphology of the flower, and to a lesser extent on the general external structure of the plant body. Even so, the arrangements of the plant groups are many and various. This multiplicity of systems is largely due to the lack of agreement as to what constitutes a primitive angiosperm. This in turn is traceable to the dearth of fossil evidence on the early history of the flowering plants. Consequently the concept of the primitive angiosperm varies with the individual phylogenist, depending upon the group of gymnosperms which is selected as the progenitor of the angiosperms. Next, various attempts have been made to establish criteria or dicta of evolution, such as BESSEY'S Dicta (**33**), HUTCHINSON'S General Principles (**83**), and ENGLER'S Prinzipien (**62**). Finally, the groups of plants have been arranged phylogenetically according to the dicta or principles of the individual creating the system.

[1] Contribution from the Biological Laboratories, Harvard University.

ENGLER and PRANTL (**60, 61, 62**) derive the Verticillatae (Casuarinaceae), the Juglandales (Juglandaceae), the Fagales (Betulaceae and Fagaceae), the Urticales (Ulmaceae, Rhoipteleaceae, Moraceae, and Urticaceae), and the Ranales in independent lines from a hypothetical group, the Protangiospermae. The Rosales are then shown as coming from the Ranales. In the Urticales, the Ulmaceae are considered to be primitive.

WETTSTEIN (**158**) derives the Verticillatae from the Gymnospermae. The Fagales and the Juglandales are considered to be rather closely related and the two groups have their origin in the Gymnospermae. The Urticales (Moraceae, Cannabaceae, Ulmaceae, Eucommiaceae, Rhoipteleaceae, and Urticaceae) are derived from the same line which produced the Fagales and the Juglandales. The Hamamelidales arose close to the Urticales. The Rosales are connected up to the former group through the Polycarpicae.

HUTCHINSON (**83**), in his phylogenetic arrangement of the dicotyledons, pictures evolution as proceeding from the Magnoliales, to the Dilleniales, to the Cunoniales, to the Rosales, and then to the Hamamelidales. The Fagales, the Casuarinales, and the Urticales (Ulmaceae, Barbeyaceae, Moraceae, Scyphostegiaceae, Urticaceae, and Cannabinaceae) are derived in independent lines from the Hamamelidales. However, some of the Urticales are shown as taking their origin from his herbaceous line. These herbaceous Urticales are connected up with the Aristolochiales, which in turn arose from the primitive Ranales through the Berberidales.

BESSEY (**31, 32, 33**) places the Ulmaceae, the Moraceae, and the Urticaceae in the Malvales. These three families are derived from the Tiliaceae. The Malvales originated from the Ranales. The Rosales also are shown as taking their origin in the Ranales. In the former order the Rosaceae are considered to have given rise to the Hamamelidaceae and allied families. The Casuarinaceae are joined up with the Hamamelidaceae. The Sapindales, according to BESSEY, had their origin in the Rosales through the Celastrales. The Sapindaceae are figured as giving rise to the Juglandaceae on the one hand, and to the Betulaceae and Fagaceae on the other.

HALLIER (79) derives the Hamamelidales from the Ranales through the intermediate group, the Anonales. The Terebinthales are derived from the Ranales. In the Terebinthales, the Rutaceae have given rise to the Terebinthaceae (including the Juglandaceae). The Terebinthaceae have given rise to the Urticaceae (broad sense) on the one hand, and to the Betulaceae, Fagaceae, and Casuarinaceae on the other hand. The Rosales are derived from the Ranales on still another line.

In addition to these great systems of classification, mention may be made of some of the systems of lesser prominence. RENDLE (125) follows ENGLER and PRANTL in the main. The Juglandales, Fagales, Casuarinales, Urticiflorae, and other Amentiferae are placed in a low position. The Urticiflorae are made up of the following families: Ulmaceae, Urticaceae, Moraceae, and Cannabinaceae. JOHNSON (91) also follows the Englerian scheme for the most part. He groups the families Ulmaceae, Moraceae, Urticaceae, and Cannabinaceae in the order Urticales. BENTHAM and HOOKER (21) place the Urticaceae (broad sense) in the artificial series Unisexuales, along with the Euphorbiaceae, Balanopseae, Platanaceae, Leitnerieae, Juglandeae, Myricaceae, Casuarineae, and Cupuliferae. WARMING and MÖBIUS (150) begin their classification of the dicotyledons with the orders Verticillatae, Querciflorae, Juglandiflorae, Saliciflorae, and Urticiflorae. The latter order contains the Ulmaceae, Urticaceae, Moraceae, and Cannabinaceae. Unfortunately, LOTSY did not reach the Urticales in his unfinished work (105).

It is apparent that there is a wide difference of opinion among the taxonomists as to the proper phylogenetic position of the Moraceae. Since this is the situation, it is only logical that the student of phylogeny should turn to explore the evidence from the other divisions of botanical science in his attempt to determine which, if any, of the existing phylogenetic systems is the more natural arrangement of the flowering plants.

The present study is an investigation of the anatomy of the Moraceae and of the several families which have been placed near them in the different systems of phylogeny. The attempt is made to use this anatomical evidence in tracing the phylogeny of this group of families. Further, there has been an endeavor to harmonize

the anatomical data with the facts of floral morphology and with the evidence from the other branches of plant science, such as cytology, paleobotany, and floral anatomy.

The anatomists have established, entirely independently of taxonomy and of systems of phylogeny, certain lines of specialization or trends of evolution in the structures of the plant stele. It is held by many anatomists (**135, 136, 63, 132, 120, 121, 159, 87, 109, 151, 108, 1, 148, 72, 73, 74**) that these evolutionary tendencies in the internal structure of plants should be of some aid to taxonomy and to phylogeny. Further, BAILEY and SINNOTT (**16**) and BAILEY (**11**) have pointed out the necessity for the study of all parts of the plant before a natural classification can be formulated. It must be made clear, however, that the anatomist is not striving to base classification on anatomical features alone. He does not argue that the anatomy of plants is necessarily more conservative than the structure of the flower or fruit. He recognizes that in evolution not all the structures of an organism may be proceeding in specialization at the same rate; thus in some groups the flower probably has evolved relatively rapidly, with the stem remaining more or less at the same evolutionary level, while in other groups the stem has become specialized with the reproductive organs remaining more or less the same. But the anatomist does feel that any truly natural classification must take cognizance of the facts of internal structure as well as those of external morphology.

These lines of structural evolution, chiefly in the secondary xylem, may be summarized briefly as follows:

1. The vessel element with a scalariform perforation plate (fig. 28) is more primitive than the element with a simple perforation plate (fig. 6) (**87, 18, 141, 142, 34, 64, 65**).

2. Vessel elements with scalariform perforation plates may have apertures with complete borders, with borders to the middle of the orifice, with borders only at the ends of the orifice, and with nonbordered apertures (fig. 28). Evolution has proceeded in the order just given (**65**).

3. In the class of vessel elements with scalariform perforation plates, the type with many (over 15) bars to a plate (fig. 57) is primitive; the type with intermediate (5–15) bars (fig. 28) comes next; and the type with few (5 or fewer) bars is highest (fig. 61) (**65**).

4. Among the vessel elements with scalariform perforation plates, the type with narrow openings (fig. 57) precedes the type with wide openings (**65**).

5. There is a decrease in length of vessel element as the vessel elements become specialized (fig. 23) (**18, 9, 64**).

The length of a vessel element is determined by two factors: (1) the length of the cambial initial from which it was derived, and (2) the amount of elongation which takes place during differentiation. BAILEY (**9**) has shown that as the secondary xylem of the gymnosperms and angiosperms becomes more specialized, the cambial initials decrease in length. Further, he demonstrated that in most cases the vessel members are equal to the cambial initials in length. The latter conclusion was also reached by CHALK and CHATTAWAY (**44**). However, BAILEY (**9**) did find that some of the more primitive types of vessel elements are slightly longer than their cambial initials, and some of the more advanced types of vessel members are slightly shorter than their cambial initials. Likewise CHALK and CHATTAWAY (**45**) report that the vessel elements in the early wood of certain ring-porous woods are considerably shorter than their cambial initials. It is clear, therefore, that the fact that the vessel elements show a gradual decrease in length in the dicotyledons is dependent on the fact that the cambial initials are decreasing in length as the woods of these plants become more specialized. The two exceptions just noted merely serve to accentuate the effects of this shortening process at the two ends of the series.

6. There is an increase in the diameter of the vessel elements as they become specialized (figs. 38, 19, 3) (**18, 9, 64**).

7. Vessel members with angular cross-sectional outline (fig. 39) are primitive; those with round outlines (fig. 26) are derived (**11, 64**).

8. Vessel elements with thin walls (fig. 39) precede those with thick walls (fig. 19) (**64**).

9. As the vessel elements become specialized, their end walls become less and less oblique until a transverse end wall is produced (figs. 57, 23) (**18, 64, 65**).

10. The evolutionary development of intervascular pitting and vessel-parenchyma pitting proceeds from scalariform (fig. 55) to transitional (fig. 54) to opposite (fig. 53) to alternate (fig. 6) (**38, 18, 64, 66**).

11. Diffuse-porous woods (fig. 4) precede ring-porous woods (fig. 3) (**64**).

12. Spiral thickenings in vessel members are evidence of specialization (**66**).

13. The solitary pore arrangement (fig. 39) is more primitive than the various aggregate arrangements of pores; that is, pore multiples (fig. 15), pore clusters (fig. 18), and pore chains (FROST, unpublished data).

14. Evolution has proceeded from tracheids (fig. 52) to fiber-tracheids (fig. 49) to libriform wood fibers (fig. 56) (**87, 18, 11, 13**).

15. There is a phylogenetic decrease in length in the fibrous tracheary elements as the tracheids, fiber-tracheids, and libriform wood fibers become more and more specialized (**18, 9, 11**).

As with the vessel elements, the length of fibrous tracheary elements is determined by two factors: (1) the length of the cambial initials from which they were derived, and (2) the amount of elongation which takes place during differentiation. It has already been stated that the length of the cambial initials decreases as specialization proceeds in the secondary xylem of the dicotyledons. CHATTAWAY (**48**) has found that the percentage of elongation of the fibers increases as the cambial initials become shorter. In other words, very long cambial initials do not elongate much in the ontogenetic differentiation of fibers, but short cambial initials may elongate as much as nine times their original length in the formation of mature fibers. Yet the amount of decrease in the length of the cambial initials is so much greater than the amount of elongation that the generalization that fibrous tracheary elements decrease in length with specialization of the wood is not upset.

16. The non-septate fiber, either fiber-tracheid or libriform wood fiber, precedes the septate fiber, either septate fiber-tracheid (fig. 2) or septate (libriform) wood fiber.

17. Heterogeneous rays are more primitive than homogeneous rays. KRIBS (**102**) has shown that the evolutionary sequence is from heterogeneous type I, the most primitive, through the transitional heterogeneous types IIA (fig. 7) and IIB (fig. 8), to homogeneous type I (fig. 10), to the highly specialized homogeneous type II rays. Further, the uniseriate homogeneous type III rays (fig. 32) are more

highly specialized than the uniseriate heterogeneous type III rays (fig. 9). Heterogeneous type III is regarded as a derivative of either heterogeneous type I or II. Homogeneous type III may have evolved from any of the other types. It appears that the uniseriate types are highly specialized structures, owing to the elimination of multiseriate rays.

The status of the so-called aggregate, diffuse, and compound rays is such a controversial matter that no final statement concerning them can be made at the present time. However KRIBS (**102**), after an extensive survey of rays in the dicotyledons, comes to the conclusion that "the so-called compound ray is merely an unusually wide multiseriate ray. Such rays are of common occurrence in both primitive and specialized types of dicotyledons, and may be of either the heterogeneous or the homogeneous type. The so-called aggregate ray is a specialization which occurs sporadically. It is an offshoot from the main line of structural specialization in rays."

18. As for xylem parenchyma, the diffuse type (fig. 38) is primitive and the various aggregations known as vasicentric (fig. 22), aliform (fig. 11), confluent (fig. 19), and metatracheal parenchyma (fig. 15) are derived (BAILEY, unpublished data; **87**).

19. BAILEY (**10, 11**) has shown that in structurally primitive woods, the fusiform initials of the cambium overlap and the increase in the circumference of the lateral meristem is due to pseudotransverse, anticlinal divisions followed by longitudinal sliding growth of these cells. Consequently the cells of the mature wood are non-stratified. This type of cambial activity culminates in a type where the anticlinal divisions of the fusiform initials are radiolongitudinal. In such stems the cambial derivatives are arranged in parallel horizontal series. Thus the storied arrangement of elements (fig. 23); that is, rays, vessel members, and even fibers and wood parenchyma strands, indicates a highly specialized stem (**116, 119, 85**).

20. Herbs have been derived from shrubs and trees (**57, 133, 134, 87, 89, 90**).

It will serve a useful purpose to refer briefly to the methods by which these salient lines of structural specialization have been worked out. In 1918, BAILEY and TUPPER (**18**) made an extensive

survey of the lengths of vessel elements and other tracheary cells in a great many living gymnosperms and dicotyledons, as well as a goodly representation of fossil Lycopsida, Sphenopsida, and Gymnospermae. They concluded that, in general, there is a tendency for the reduction in length of the tracheary cells (vessel elements, tracheids, fiber-tracheids, and libriform wood fibers), proceeding from the lower groups such as the Lycopsida, Sphenopsida, and Cycadofilicales, to the Cordaitales, Bennettitales, and Cycadales, to the Coniferales and Gnetales, and thence to the dicotyledons. This decrease in length continues in the dicotyledons. Further, the evolutionary specialization of the vessel element proceeds hand in hand with this reduction in length of the tracheary cells. That is, a phylogenetic study indicates that as the tracheary elements become shorter and shorter, the vessel members pass from the scalariform to the simple perforate state, they become wider, the pitting on their lateral walls changes from scalariform to transitional to opposite to alternate, and their end walls become less and less tapering. That the appearance and subsequent evolution of the vessel are correlated with a decrease in cell size is emphasized by the fact that the vesselless angiosperms, *Trochodendron* and *Drimys*, have the longest tracheary elements of any of the dicotyledons investigated by BAILEY and TUPPER. Furthermore, the tracheary elements of the Gnetales which possess vessels resemble in size those of many dicotyledons. Finally, correlated with this decrease in size and with this vessel evolution, the fibrous tracheary elements become increasingly specialized; that is, the bordered pits become smaller and smaller, until ultimately a libriform wood fiber with simple pits is produced.

FROST and KRIBS, using the research of BAILEY and TUPPER as a basis, found that the specialization of other features of the vessel members and of the rays correlated to a remarkable degree with the decrease in size of the tracheary cells.

From this brief description of the methods of the anatomist, it can be seen that these lines of specialization are not based on any system of angiosperm classification, but have been formulated independent of any preconceived notion that this or that group of gymnosperms has given rise to the angiosperms; or any preconceived idea that the Ranales or the Amentiferae are primitive. Con-

sequently any suggestions which the anatomist makes as to phylogeny will be unbiased, and therefore of correspondingly greater value than would be the case were not his methods founded independent of classification.

Material and methods

Slides of the secondary xylem from the stems of 465 species, representing 165 genera and 22 families, were examined in detail during this investigation. These families (defined by ENGLER and PRANTL (**61, 62**) unless otherwise indicated) include the Moraceae, Ulmaceae, Urticaceae, Rhoipteleaceae (**60**), Hamamelidaceae, Eucommiaceae, Platanaceae, Myrothamnaceae, Buxaceae, Stachyuraceae, Casuarinaceae, Betulaceae, Fagaceae, Juglandaceae, Hydrangeaceae (family by HUTCHINSON, **83**; equivalent to the subfamily Hydrangeoideae of the Saxifragaceae of ENGLER and PRANTL), Grossulariaceae (family by HUTCHINSON, **83**; equivalent to the subfamily Ribesioideae of the Saxifragaceae of ENGLER and DIELS, **60**), Escalloniaceae (family by Hutchinson, **83**; equivalent to the subfamily Escallonioideae[2] in the Saxifragaceae of ENGLER and PRANTL, **61**), Brunelliaceae, Cunoniaceae, Dichapetalaceae (or Chailletiaceae), Rosaceae, and Calycanthaceae. In addition, all available slides of the Aristolochiaceae, Sterculiaceae, and Tiliaceae were studied, but no detailed descriptions were made of these groups since anatomically they were found not to be closely related to the Moraceae.

The material of the Moraceae was collected in various parts of the world by many different collectors. The major part of the collection was assembled at the Yale School of Forestry. It was through the courtesy of Professor SAMUEL J. RECORD of Yale University that most of the woods were made available for the present study.

Following the technique outlined by BAILEY (**4**), JEFFREY (**88**), and WETMORE (**156**), small blocks of the woods were boiled, cooled, and then softened in hydrofluoric acid. Next the material was washed, dehydrated, and stored in glycerin-alcohol. Later transverse, radial, and tangential sections were cut with a Jeffrey-

[2] In the second edition of ENGLER and PRANTL (**62**), four other sub-families are split off from this one sub-family.

Thomson sliding microtome. These sections, 10–15 μ in thickness, were stained in Heidenhain's iron-alum haematoxylin and then counter-stained in safranin.

The xylem of the other families was studied from slides in the extensive Harvard collection.

Eight or nine slides of each species were available in the material of the Moraceae. Often two or more representatives of the same species from different geographical localities were studied. In the other families, more than one slide of each species was studied whenever possible, and frequently specimens of the same species from different parts of the world were examined microscopically.

The terms used in the anatomical descriptions of the families which follow this section are those approved by the Committee on Nomenclature of the International Association of Wood Anatomists (**50**). These terms have been further elaborated and illustrated by RECORD, the chairman of the Committee (**119**). After the appearance of these two publications on terminology, BAILEY (**13**) gave more practical definitions of the terms tracheid and fiber-tracheid. His usage of the two terms is followed in this study; that is, fiber-tracheids are elements with pits with much reduced or vestigial borders—conspicuously smaller than the corresponding pits on the vessel members (fig. 49)—and tracheids are elements with large bordered pits (equal in size to the pits on the vessel elements) (*cf.* figs. 52 and 53).

FROST (**65**) has been followed in classifying scalariform perforation plates as to the number of bars; that is, the plates with 15 bars or more are placed in the many class, those with 5 to 15 bars are placed in the intermediate class, and the plates with 5 or fewer bars in the few class. Further, the scalariform perforation plates with openings of 6 μ (measured from bar to bar) or less are described as narrow; those with apertures of more than 6 μ are designated wide.

KRIBS' (**102**) classification of rays is used in this investigation. Ray width and ray depth do not appear to have much phylogenetic significance; indeed it is questionable whether they are of any great diagnostic value, for they exhibit considerable variation, not only from tree to tree, depending on site, but at different levels in the same tree, and at various distances from the pith at the same level (**8, 111, 55, 152**). However, a brief notation on the range of varia-

tion in ray width and depth is included in the anatomical description of each family.

Whenever the anatomical description of a family was, by necessity, based on material cut from a region close to the pith, this fact is indicated. The ideal situation, of course, would be one where it would be possible always to compare the homologous parts of the plants of all the families, for it has been shown by BAILEY and FAULL (**14**) that in a given species the range of variability tends to be greater in different parts of a single, large mature tree than in homologous parts of different trees. Since this ideal arrangement is not always possible to attain, the next best thing is to record any youthful material employed in an investigation so that a correction may be made in the interpretation of any conclusions based on this young material. Many investigators (**130, 15, 115, 18, 101, 42**) have shown that from the pith outward, the lengths of vessel elements and of other tracheary cells increase very rapidly for a number of years, and then remain at about the same relative length, but fluctuating slightly with changes in environmental conditions. Vessel diameter also increases from the pith out to the bark (**115, 9**). Multiseriate ray width increases centrifugally from the pith (**55**). Multiseriate ray depth seems to decrease from the pith outward (BAILEY, unpublished data). Further, plants with homogeneous rays in the mature wood may have heterogeneous rays in the wood near the pith (figs. 33–36). Thus it seems that there is an increase in the homogeneity of the rays from the pith out to the cambium. Enough has been said about variation from youth to old age in wood to demonstrate the importance of using homologous regions in comparative anatomical studies. At least, if this ideal is not always attainable, any departures from the use of homologous regions should be recorded.

Rough measurements of vessel diameter and vessel element length are given for each family. These figures were derived in the following manner: Only one species of each genus was measured. For the vessel diameter (tangential) figures, 25 vessels, selected at random, were measured from sections with a graduated ocular disc calibrated against a stage micrometer. These measurements were averaged and the resulting mean was then averaged with the means of other genera in computing the mean for the family. The measurements of vessel element length were treated in the same way, except

that only 12 vessel members were measured from sections. The total body length of the vessel element (that is, the extreme tip of one end to the tip of the other) was measured, for it has been shown that total body length, rather than body length or extreme body length, corresponds more nearly to the length of the cambial initial from which the vessel element was derived (**44**). In ring-porous woods the elements in the late wood were measured, for their length is nearer that of the cambial initials than are the vessel members in the early wood (**45**). During differentiation the vessel elements in the latter region undergo considerable shortening, probably owing to the great lateral swelling of the vessel members in this zone. CHALK (**43**) has set up certain size classes for vessel element length, and in recording the present measurements, the size classes are indicated. These size classes are defined as follows:

SHORT	
Extremely short	less than 177 μ
Very short	177–240
Moderately short	240–342
MEDIUM SIZED	
Medium sized	342–799
LONG	
Moderately long	799–1131
Very long	1131–1866
Extremely long	over 1866

The size classes employed for the vessel diameter figures are those of CHATTAWAY (**47**). The limits of these classes are as follows:

Extremely small	up to 30 μ
Very small	30–50
Small	50–100
Moderate sized	100–200
Rather large	200–300
Large	300–400
Very large	over 400

Of course these measurements on the vessel elements are of the crudest sort. In the first place, the method of measuring vessel element length on sections gives only approximate lengths, for often the "tails" or "ligules" of vessel members elude the observer.

Macerated material should be used for best results. In the second place, too few measurements for each species were made. In the third place, these figures in no way represent the mean for the species, for it has been shown by RENDLE and CLARKE (**126, 127**) that the variations in cell size in wood are of 4 types: (a) Local variation in a small sample owing to differences in development of the various cells from the cambium. (b) Variations from the pith to the periphery. (c) Variations from the base of the stem upward (**130, 42, 54**). (d) Variations resulting from differences in external growth conditions (**113**). Therefore, to secure a mean for a species, accurate within $\pm$ 10 per cent of the actual mean (for vessel diameter, for example), RENDLE and CLARKE (**126**) have shown that it is necessary to measure 25 vessels selected at random from each of 5 samples taken from each of 4 trees. Of course such extensive measurements are prohibitory in an investigation of this character. The figures here are not meant to represent anything but very rough estimates of the cell size in the various families. Alone they mean nothing, but where they correlate with all the morphological data it would appear that they have some significance. Under the circumstances, to record the standard deviation and standard error would be most misleading. DESCH (**53**) has said, "It is important to bear in mind that data collected by bad sampling will become no more illuminating by the calculation of standard errors; such treatment may merely lend spurious support to false conclusions."

The fibrous tracheary elements were not measured, for it was found that these cells could not be measured very accurately in sections; that is, macerated material gave much higher values.

Anatomical descriptions of the families

MORACEAE[3]

The Moraceae are largely a tropical family with but few representatives in temperate regions. The family is largely arboreal or

[3] Anatomical descriptions of this and of the other families studied are given by SOLEREDER (**136**), RECORD and MELL (**124**), MOLL and JANSSONIUS (**110**), and DADSWELL and RECORD (**52**). The anatomy of various genera and species of the Moraceae and of the other families is described by RECORD (**119, 122**), KANEHIRA (**95, 96**), TANG (**138, 139**), BROWN and PANSHIN (**40**), JONES (**94**), BROWN (**39**), JEFFREY (**87**), GARRATT (**71**), RECORD and GARRATT (**123**), TUPPER (**146**), KOEHLER (**99**), and GAMBLE (**70**).

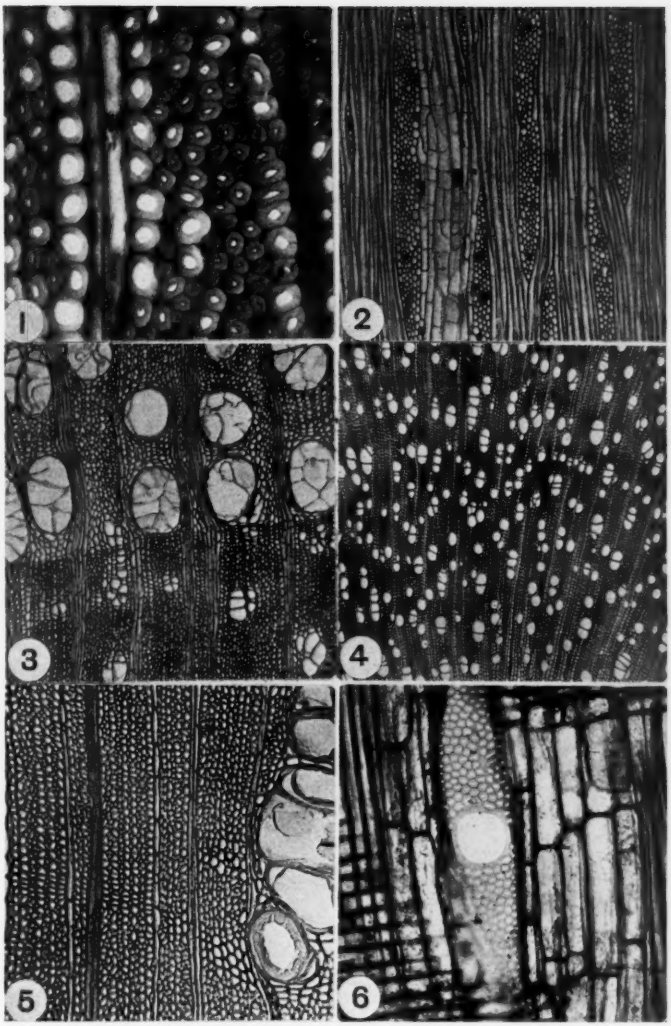
shrubby, with very few herbaceous forms. According to ENGLER and PRANTL (**61**), there are 73 genera and about 1000 species in the Moraceae. Fifty genera and 100 species were examined in this investigation. The genera studied include: *Trophis* (3 spp.), *Morus* (2 spp.), *Pseudomorus*,[4] *Paratrophis*, *Pseudostreblus*, *Maclura*, *Chlorophora* (2 spp.), *Bagassa*, *Malaisia*, *Plecospermum*, *Broussonetia* (2 spp.), *Taxotrophis*, *Streblus*, *Phyllochlamys*, *Sloetia* (2 spp.), *Trymatococcus* (2 spp.), *Craterogyne* (young material), *Helianthostylis*, *Sorocea* (2 spp.), *Clarisia* (2 spp.), *Cudrania* (3 spp.), *Parartocarpus* (2 spp.), *Artocarpus* (3 spp.), *Perebea* (2 spp.), *Helicostylis* (3 spp.), *Castilloa* (4 spp.), *Olmedia* (2 spp.), *Pseudolmedia* (3 spp.), *Antiaris* (3 spp.), *Brosimum* (4 spp.), *Piratinera* (4 spp.), *Bosquiea*, *Ficus* (8 spp.), *Sparattosyce*, *Musanga*, *Myrianthus* (3 spp.), *Coussapoa* (4 spp.), *Pourouma* (3 spp.), *Cecropia* (3 spp.), *Cannabis* (herb), *Humulus* (herb), *Brosimopsis*, *Gymnartocarpus*, *Poulsenia*, *Prainea* (2 spp.), *Anonocarpus*, *Noyera*, *Olmedioperebea*, *Ogcodeia* (3 spp.), and *Mesogyne*.

Most of the species do not have growth rings; in fact, only 18 species were found with them.

Forty-four of the species have fiber-tracheids (fig. 1) predominantly, but the borders on the pits of the fibers are very small. Twelve of these species have septate fiber-tracheids (fig. 2), 8 species with septate fiber-tracheids alone and 4 with but a few septate fiber-tracheids among the non-septate fiber-tracheids. Fifty-four of the species have libriform wood fibers (fig. 56) predominantly. Sixteen of these species have septate wood fibers; 11 with septate wood fibers alone and 5 with but a few septate wood fibers.

Vessel distribution is mostly a combination of solitary pores and pore multiples, with the percentage of solitary pores high compared with the percentage of pore multiples. Some forms have pore clusters (fig. 3) and very few have pore chains, in addition to the other types. Most of the species are diffuse-porous (fig. 4); only 7 species are ring-porous (fig. 3). The vessels are usually (79 spp.) round (fig. 3) in cross section; some (13 spp.) are angular and a few (7 spp.) are round to angular. Most of the species (74) have thin-walled vessels;

[4] Only one species of each genus was studied in this family and in the other families unless otherwise indicated.

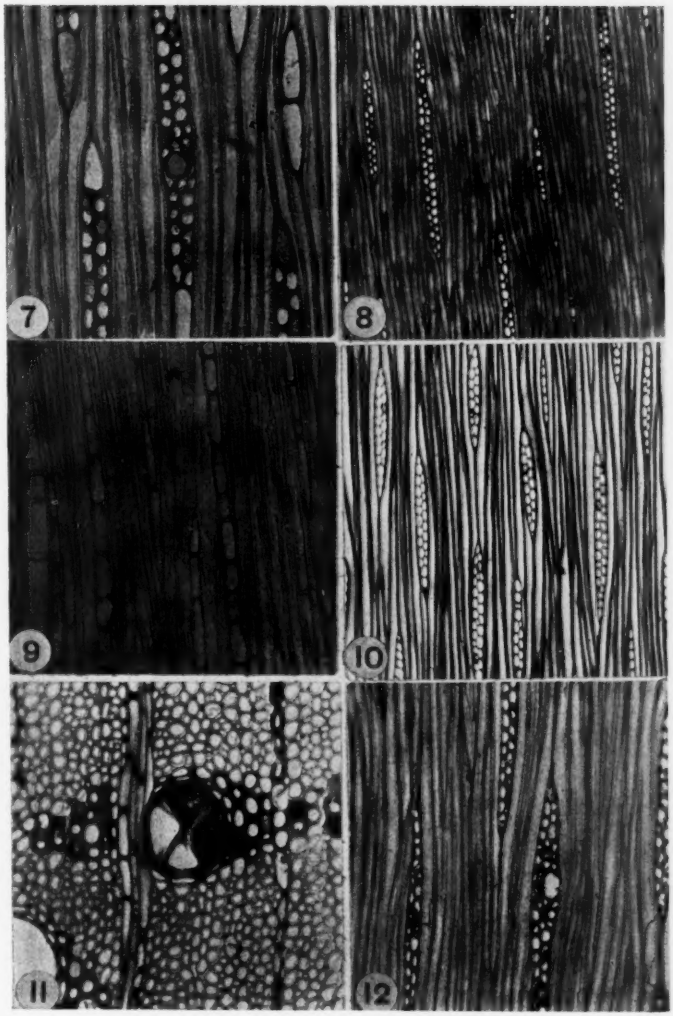


FIGS. 1–6.—Moraceae: Fig. 1, *Pararlocarpus venenosa* Becc.; transverse section. ×245. Fig. 2, *Antiaris toxicaria* Lesch.; tangential section. ×40. Fig. 3, *Morus rubra* L.; transverse section. ×45. Fig. 4, *Cannabis sativa* L.; transverse section. ×25. Fig. 5, *Trymatococcus turbinatus* Ducke.; transverse section showing latex tube in a ray. ×100. Fig. 6, *Trophis macrostachya* Donn.; radial section. ×150.

about a fourth (25 spp.) have vessels with thick walls. The vessel diameters are very small (fig. 4) to large (fig. 3) (averages range 40 to 332 μ; mean for the family 127 μ). Tyloses are present in most (77) species. These may be few in number (20 spp.) or many (57 spp.) (fig. 3). Most (68) of the species with tyloses have thin-walled tyloses (fig. 3); some (9 spp.) have sclerotic tyloses (fig. 5) with ramiform pits. All the vessel elements have simple perforation plates (fig. 6). The angle which the end walls of the vessel members make with the lateral walls varies from 20° to 90°. Only 13 species have end walls at 90° and then only a few of the end walls are 90°. The intervascular pitting in all is alternate and crowded (fig. 6). The vessel elements are extremely short to medium sized (averages range 125 to 501 μ; mean of the family 335 μ). There are spiral thickenings in some, or in all of the vessel elements of 9 species—all the forms with ring-porous woods and two other species.

As for ray type, 13 species have heterogeneous I rays, 26 have heterogeneous IIA rays (fig. 7), 53 have heterogeneous IIB rays (fig. 8), 1 has heterogeneous III rays (young material however) (fig. 9), and 6 have homogeneous I rays (figs. 10, 2, 12, 13). In width, the rays vary from 1 to 15 cells. In depth, the uniseriate rays vary from 1 to 57 cells high; the multiseriate rays from 2 to 192 cells high. A number of species (18) have rays which contain a few sheath cells. Ten species have rays with some sclerotic ray cells. Often the forms with sclerotic tyloses have thick-walled fibers, thick-walled vessels, sclerotic ray cells, and sclerotic xylem parenchyma cells (fig. 11). Table 1 shows this correlation.

There are latex tubes in 38 species (fig. 7). These tubes are not intercellular canals but are long laticiferous cells with very obvious cell walls. The latex tubes in the rays are continuous with those in the pith and phloem (fig. 14). Figure 5 shows a latex tube in a transverse section of xylem. The lack of cross walls in the tube is apparent. Similar laticiferous cells have been described in the Apocynaceae (**160**). The latex tubes in the Moraceae may vary from small (fig. 2) to large (fig. 12). There may be few (10 spp.) or many (28 spp.) in the xylem. They are usually in the center of the ray (fig. 13), rarely near the top; only in one species, *Craterogyne kameruniana* (Engl.) Lanj. (the form with only uniseriate, or hetero-



FIGS. 7–12.—Moraceae: Fig. 7, *Trymatococcus amazonicus* Poepp. and Endl.; tangential section showing latex tube. ×200. Fig. 8, *Sloetia sideroxylon* Teijsm. and Binn.; tangential section. ×75. Fig. 9, *Craterogyne kameruniana* (Engl.) Lanj.; tangential section. ×100. Fig. 10, *Gymnartocarpus woodii* Merrill.; tangential section. ×50. Fig. 11, *Helicostylis tomentosa* Rusby; transverse section showing sclerotic xylem parenchyma cells, sclerotic ray cells, and sclerotic tyloses. ×120. Fig. 12, *Parartocarpus venenosa* Becc.; tangential section showing latex tube. ×85.

geneous III rays), are some of the latex tubes outside of the rays. No latex tubes were observed in the sub-families Conocephaloideae and Cannaboideae.

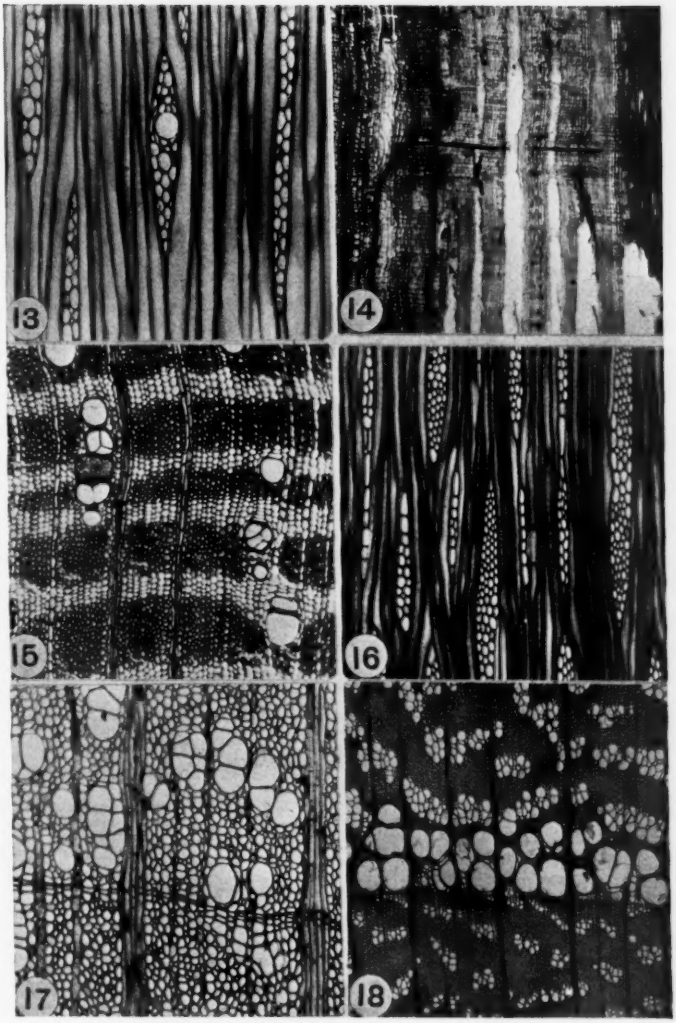
The xylem parenchyma distribution is mostly a combination of aliform and confluent (32 spp.) or metatracheal (24 spp.) (fig. 15).

TABLE 1

CORRELATION BETWEEN PRESENCE OF SCLEROTIC TYLOSES AND PRESENCE OF OTHER THICK-WALLED ELEMENTS

NAME OF PLANT	SCLEROTIC TYLOSES	FIBER WALL	VESSEL WALL	SCLEROTIC RAY CELLS	SCLEROTIC WOOD PARENCHYMA CELLS
Trymatococcus turbinatus (Baill.) Ducke	+	Fairly thin	Thin	−	−
Cudrania spinosa Tréc.	+	Very thick (hardly any lumen)	Thick	−	−
Cudrania javanensis Tréc.	+	Very thick (hardly any lumen)	Thick	+ (few)	+ (many)
Helicostylis tomentosa (P. & E.) Rusby	+	Fairly thick	Thin	+ (quite a few)	+ (many)
Pseudolmedia spuria (Sw.) Gris.	+	Very thick (hardly any lumen)	Fairly thick	+	+
Piratinera guianensis Aubl.	+	Thick	Fairly thin	+ (many)	+ (few)
Piratinera scabridula Blake	+	Very thick (hardly any lumen)	Fairly thin	+ (many)	+ (few)
Piratinera velutina Blake	+	Thick	Thick	+ (many)	+ (all)
Piratinera panamensis Pittier	+	Thick	Thick	+	−

In the latter type the bands may vary from 1 to 22 cells in width. In other species there are various combinations of vasicentric, aliform, confluent, and metatracheal. Only 4 species have terminal parenchyma. None of the species examined shows either diffuse or the absence of xylem parenchyma. *Maclura aurantiaca* Nutt. is the only form with fusiform wood parenchyma cells. Seven species have sclerotic xylem parenchyma cells (fig. 11).



FIGS. 13–18.—Fig. 13, *Parartocarpus triandrus* J. J. Smith, Moraceae; tangential section showing latex tube in a ray. ×90. Fig. 14, *Ficus populoides* Warb., Moraceae; radial section showing latex tube in pith (left) bending out through xylem (center) to phloem (right). ×25. Fig. 15, *Malaisia scandens* Planch., Moraceae; transverse section. ×55. Fig. 16, *Ficus benghalensis* L., Moraceae; tangential section. ×65. Fig. 17, *Planera aquatica* J. F. Gmel., Ulmaceae; transverse section. ×80. Fig. 18, *Ulmus americana* L., Ulmaceae; transverse section. ×25.

There is a tendency toward storied structure in *M. aurantiaca* and *Ficus benghalensis* L. (fig. 16).

The vessel-parenchyma pitting is largely (90 spp.) alternate. Only 6 species have both opposite and alternate pitting. Seventy-six species have some unilateral compound pitting.

Very often there are crystals in the ray cells, or in the wood parenchyma cells, or both. Frequently there are chains of chambered parenchyma cells bearing crystals among the wood parenchyma cells, or among the upright cells of the rays, or both.

Two herbs, *Cannabis sativa* L. (fig. 4) and *Humulus japonicus* Sieb. and Zucc., were examined. Internodal transverse sections reveal the fact that both of these plants are of the "continuous" herb type (that is, the secondary wood forms a complete ring and is not broken up into a number of discrete bundles as in *Aristolochia* or *Clematis*). At the nodes the solid rings are slightly broken up in places, owing to the fact that the leaf traces are flanked by thin layers of parenchyma.

In general it may be said that anatomical specialization has proceeded from the Moroideae to the Artocarpoideae to the Conocephaloideae to the Cannaboideae. However, there are two complicating factors in the anatomical evolution of this family. First, although most of the members of the Moroideae are primitive as far as most anatomical characters are concerned, certain temperate members are rather highly specialized in a number of vessel characters. About a third of the species examined in the Moroideae have some vessel elements with end walls at 90°, a much higher percentage than was found in the other sub-families, excluding the Cannaboideae. Further, in the Moroideae there are many more woods with thick-walled vessels and with ring-porosity than in the other sub-families. Also vessel element length is less than this length in all the sub-families except the Cannaboideae. Thus, in the subfamily Moroideae there are many anatomically primitive woods as well as some of the most highly specialized woods in the family. The explanation for this situation seems to lie in the fact that all except one of the temperate, ring-porous woods of the family are in the Moroideae. An examination of these ring-porous woods, as well as those of other families, reveals that many other characters

in these woods are specialized, even though the nearest relatives are quite primitive anatomically. Table 2 contains a summary of the chief characters of the ring-porous woods of the Moraceae. All the species listed, with the exception of *Cudrania pubescens* Tréc., are in the Moroideae. It can be seen from this table that in these ring-porous species the vessel elements are exceedingly short to very short, the vessels are round in transverse section, some vessel mem-

TABLE 2

DATA ON RING-POROUS WOODS OF THE MORACEAE

NAME OF PLANT	VESSEL ELEMENT LENGTH (μ)	SHAPE OF VESSEL IN CROSS SECTION	THICKNESS OF VESSEL WALL	END WALL OF VESSEL ELEMENTS	SPIRAL THICKENINGS IN VESSEL ELEMENTS	RAY TYPE	TYPE OF FIBROUS TRACHEARY ELEMENT
Morus alba L.	178	R*	Some fairly thick; most thin	Few with 90°	+	Heterog. IIB	L. w. f.*
Morus rubra L.	161	R	Thin	Some with 90°	+	Heterog. IIB	L. w. f.
Maclura aurantiaca Nutt.	154	R and A*	Thick	Some with 90°	+	Heterog. IIB†	L. w. f.
Plecospermum spinosum Tréc.	231	R	Thick	Few with 90°	+	Homog. I	L. w. f.
Broussonetia papyrifera (L.) Vent.	214	R	Thin	Few with 90°	+	Heterog. IIB	L. w. f.
Broussonetia kazinoki Sieb.	233	R	Thin	Some with 90°	+	Heterog. IIB	L. w. f.
Cudrania pubescens Tréc.	155	R	Thick	Some with 90°	+	Heterog. IIB†	L. w. f.

* R, round; A, angular; L.w.f., libriform wood fibers.

† Almost homogeneous I.

bers in each species have end walls at 90°, the vessel elements contain spiral thickenings, the rays are of a high type for the family, and all the fibers are of the highest type. This matter will be discussed later in greater detail, but at this point it may be said that it appears as though the advent of ring-porosity, or of some factor (or factors) causing ring-porosity, gives an impetus to the evolution of certain vessel features as well as to the evolution of other xylary structures. Thus on the whole the Moroideae are the most primitive sub-family in the Moraceae, yet the development of ring-porosity in certain species has led to a general speeding-up of evolutionary development in the xylem of these forms, so that they are more

highly specialized than some of the species in the higher sub-families.

The second complication appears in the sub-family Cannaboideae. The fact that all the plants in this group are herbs and the fact that in almost all anatomical characters this sub-family is higher than the other sub-families, would indicate that on anatomical grounds the Cannaboideae are the highest sub-family in the Moraceae. But the Cannaboideae appear to be primitive as far as a few characters are concerned; that is, the vessels in cross section are angular to round, the vessel diameter is smaller than in the other sub-families, and all the rays are of the lowest type—heterogeneous I. This situation clears, however, when it is remembered that these plants are all herbaceous and that the xylem of an herb is essentially the first annual ring of the plant (**133, 134**). In other words, the xylem in herbs is not homologous to the xylem taken some distance from the pith of trees. The xylem of herbs is essentially "young material" and is subject to the dangers of interpretation reviewed in the introduction of this study. It is not surprising therefore to find in the otherwise highly specialized and herbaceous Cannaboideae, that the vessels are angular to round in cross section, that the vessel diameters are low, and that the rays are heterogeneous I. If these herbs were to live year after year, adding growth layers each year, one might well expect to find round pores with large diameters and rays of a high type in the hypothetical mature wood of these plants.

Before passing to a consideration of a few general conclusions based on a study of the tribes of the Moraceae, the dispersal of septate fibrous tracheary elements in the family should be given brief attention. The sub-family Artocarpoideae are the center of development of septate fibers, for 10 of the 12 species with septate fiber-tracheids and 15 of the 16 species with septate wood fibers belong in this sub-family. There are no septate fibers in the Moroideae if one excludes the genus *Mesogyne*. Likewise there are no septate fibers in the Conocephaloideae if one excepts the genus *Prainea*. There are no septate fibers in the Cannaboideae.

As for the tribes of the Moraceae, the following conclusions, based on the species examined, may be stated: There are no septate fibers

in the Fatoueae, Moreae, Broussonetieae, Strebleae, Euartocarpeae, Brosimeae, and Cannaboideae. The Dorstenieae have no septate fibers in any of the genera with the exception of *Mesogyne* previously mentioned. All the Olmedieae have some septate fibers. The Ficeae have no septate fibers except for one species, *Ficus religiosa* L. The situation with regard to septate fibers in the Conocephaloideae has already been stated. All the Dorstenieae have fiber-tracheids except *Mesogyne*. Most of the Euartocarpeae have fiber-tracheids; one species, *Brosimopsis diandra* Blake, was found with libriform wood fibers. All the Brosimeae, Strebleae, Ficeae, and Cannaboideae have libriform wood fibers. There are no latex tubes in the Fatoueae, Moreae, Strebleae, Conocephaloideae, and Cannaboideae.

It can be seen that the anatomy of the Moraceae is of rather a high type, for the vessel members have simple perforation plates; more than half of the species have libriform wood fibers; the intervascular pitting is alternate; the xylem parenchyma distribution is aliform, confluent, or metatracheal; the vessel members are short and fairly wide; and most of the vessels are round in cross section. On the other hand, the family is predominantly arboreal in habit.

This description of the anatomy of the Moraceae is in substantial agreement with the accounts in the books and papers cited at the beginning of this description, as well as with the work of BOULTON and PRICE (**37**).

ULMACEAE

The Ulmaceae are distributed in both tropical and temperate regions but mostly in the north temperate zone. There are trees and shrubs in this family but no herbs. According to ENGLER and PRANTL (**61**) there are 14 genera and about 140 species. Nine genera and 29 species were examined in the present study. The genera investigated are *Phyllostylon*, *Holoptelea*, *Ulmus* (12 spp.), *Planera*, *Celtis* (9 spp.), *Ampelocera*, *Zelkova* (2 spp.), *Trema* (young material), and *Aphananthe*.

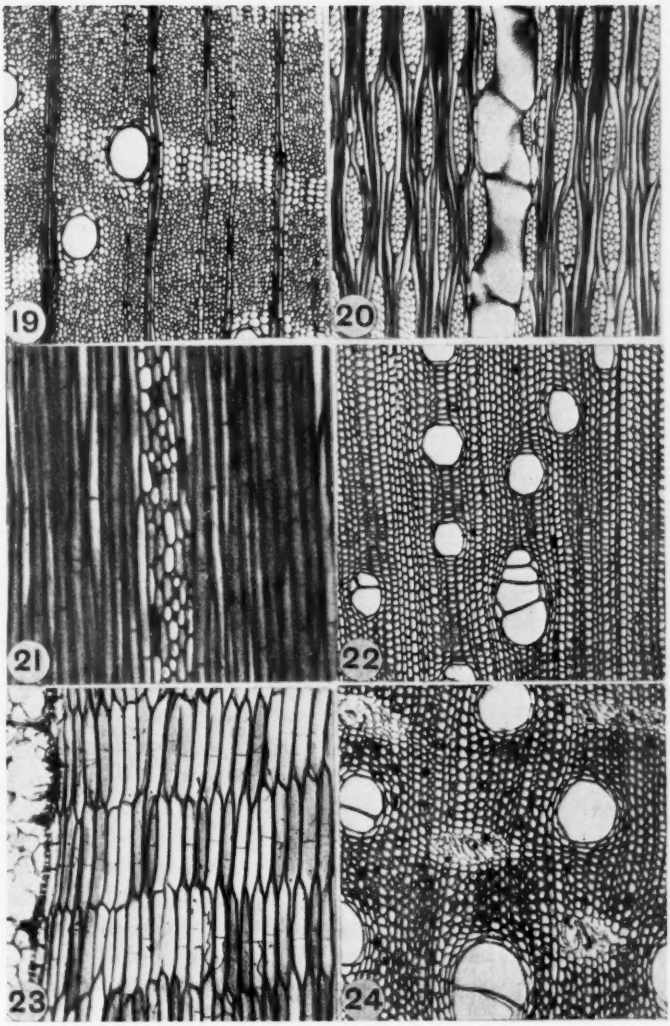
All but two of the species have growth rings (fig. 17).

The fibrous tracheary elements are either fiber-tracheids or libriform wood fibers; if fiber-tracheids, the borders of the pits are small. Four species have fiber-tracheids (fig. 49), 1 species has both fiber-

tracheids and libriform wood fibers, and 24 species have libriform wood fibers. There are no septate fibers of any character in the species examined.

Vessel distribution is a combination of solitary pores, pore multiples, and pore clusters (figs. 17, 18), or a combination of solitary pores and pore multiples. In either case the percentage of solitary pores is always greater than the percentage of pore multiples Thirteen of the species are diffuse-porous (fig. 17) and 16 species are ring-porous (fig. 18). Two species have angular (fig. 17) vessel outlines in cross section, 9 have angular and round (fig. 19), and 10 have round. Most of the species (15) have thin vessel walls (fig. 17), others (2 spp.) have thin and thick walls, and a few (4 spp.) have thick walls. The vessel diameters are very small (fig. 17) to rather large (fig. 18) (averages range 37 to 166 μ; mean of the family 96 μ). Tyloses are present in only a few (6) species (fig. 18). In these species the tyloses are many and thin-walled. Ten of the species have vessel elements with simple perforation plates and with some of the vessel elements bearing vestigial scalariform perforation plates (fig. 50). Twelve species have vessel elements with exclusively simple perforation plates. The angle which the end walls of the vessel members make with the lateral walls (fig. 20) varies from 20° to 90°. Eleven species have a few vessel elements with end walls at 90°; 10 species have no end walls at 90°. The intervascular pitting is opposite and alternate in 3 species and alternate (fig. 20) in 18 species. In both groups of species the pitting is crowded. The vessel elements are extremely short to medium sized (averages range 185 to 451 μ; mean of the family 286 μ). Most of the species (21) have spiral thickenings in some or in all of the vessel elements. As in the Moraceae, all the ring-porous species (16) have spiral thickenings. In addition, 3 species with a tendency toward ring-porosity and 2 species with diffuse-porous wood have spiral thickenings.

As for ray type, 7 species have heterogeneous I rays, 4 species have heterogeneous IIA rays, 5 species have heterogeneous IIB rays (fig. 20), and 13 species have homogeneous I rays. The ray width varies from 1 to 20 cells. In depth, the uniseriate rays run from 1 to 27 cells high and the multiseriate rays from 4 to 160 cells high. A few forms (5 spp.) have sheath cells in the rays.



FIGS. 19–24.—Fig. 19, *Aphananthe aspera* Planch., Ulmaceae; transverse section. ×55. Fig. 20, *Holoptelea integrifolia* Planch., Ulmaceae; tangential section showing storied arrangement. ×60. Fig. 21, *Myriocarpa magnifica* Rusby, Urticaceae; tangential section. ×90. Fig. 22, *Boehmeria macrophylla* Hornem., Urticaceae; transverse section. ×70. Fig. 23, *Urera baccifera* Gaudich, Urticaceae; tangential section showing storied arrangement. ×70. Fig. 24, *Gyrotaenia myriocarpa* Griseb., Urticaceae; transverse section showing island type of unlignified areas of xylem parenchyma. ×70.

Usually the xylem parenchyma distribution is either a combination of terminal and vasicentric arrangements (6 spp.) (fig. 18), or is aliform and confluent (4 spp.) (fig. 19). In other species the parenchyma distribution may be various other combinations of the preceding types and of the metatracheal arrangement. In only 1 species, *Planera aquatica* (Walt.) J. F. Gmel. (fig. 17), is the parenchyma diffuse and vasicentric.

There is a tendency toward storied structure in 3 species and well developed seriation of rays and vessel elements in 2 species (fig. 20).

The vessel-parenchyma pitting is opposite and alternate in 3 species, and alternate in 25 species. Only 1 species has unilateral compound pitting.

Very often there are crystals in the ray cells, or in the wood parenchyma cells, or in both. One species has crystalliferous chambered parenchyma cells among the xylem parenchyma strands.

On the whole the Ulmaceae seem more primitive anatomically than the Moraceae. In the former there are no herbs and no septate fibers. Half of the species have vessels with angular, or angular and round outlines in cross section. In general the vessel diameters are smaller than in the Moraceae. There is some opposite and alternate intervascular pitting in the Ulmaceae, whereas it is invariably alternate in the Moraceae. Lastly and most important of all, in the Ulmaceae some of the species have vestigial scalariform perforation plates in addition to the simple type. This would indicate that the Ulmaceae are more closely allied than are the Moraceae to some group with scalariform perforation plates on the vessel elements. The work of BAILEY and TUPPER (**18**) and FROST (**64, 65**) indicates that the type of perforation plate on the vessel members is a very strong phyletic character. There is no evidence that this character is readily modified by changes in the environment.

On the other hand, the Ulmaceae seem to be higher than the Moraceae as far as certain vessel characters are concerned. There are many more ring-porous woods in the Ulmaceae. Half of the species have some vessel elements with the end walls at 90°. The length of the vessel members is less in the Ulmaceae. More than half of the species have spiral thickenings. Most of the woods have libriform wood fibers. Over a third of the species have homogeneous

I rays. There is slightly more storied structure in the Ulmaceae than in the Moraceae.

The key to this situation seems to be the comparatively large number of ring-porous forms in the Ulmaceae. It will be remembered that in the Moraceae, the species with ring-porous woods were much higher anatomically than their immediate diffuse-porous relatives. It appears that the advent of ring-porosity, or some factor (or factors) causing ring-porosity, gives impetus to anatomical specialization. So the Ulmaceae appear to be a group which is more primitive

TABLE 3

DATA ON RING-POROUS WOODS OF THE ULMACEAE

NAME OF PLANT	VESTIGIAL PERFORATION PLATES	VESSEL ELEMENT LENGTH (μ)	SHAPE OF VESSEL IN CROSS SECTION	THICKNESS OF VESSEL WALL	END WALL OF VESSEL ELEMENTS	SPIRAL THICKENINGS IN VESSEL ELEMENTS	RAY TYPE	TYPE OF FIBROUS TRACHEARY ELEMENT
Ulmus americana L.	+	237	R*	Thin	Few with 90°	+	Homog. I	L.w.f.*
Ulmus mexicana Planch.†	+	243	R	Thin	None with 90°	+	Homog. I	L.w.f.
Celtis occidentalis L.	+	298	A* and R	Thin	Few with 90°	+	Heterog. IIB	L.w.f. and F.t.* (some)
Celtis sinensis Pers.	+	300	R and A	Thin	Few with 90°	+	Heterog. I‡	L.w.f.
Zelkova serrata Makino	−	185	R and A	Thin	None with 90°	+	Heterog. IIA	F.t.

* R, round; A, angular; F.t., fiber-tracheids; L.w.f., libriform wood fibers.
† Tendency toward ring-porosity. ‡ Almost heterogeneous IIA.

than the Moraceae in many ways; yet seemingly because this family is largely in the temperate region and has developed ring-porosity, the evolution of many other characters has been speeded up.

It is significant that when the ring-porous forms of the Ulmaceae are compared with those of the Moraceae, the latter are higher anatomically than are the former (*cf.* tables 2 and 3). Likewise when the diffuse-porous woods of the two families are compared, the woods of the Ulmaceae are found to be more primitive. In other words, the fact that the proportion of ring-porous to diffuse-porous woods in the Ulmaceae is higher than in the Moraceae seems to explain why the mean vessel element length is shorter and why there are a

larger number of forms with vessel element end walls at 90°, with libriform fibers, with homogeneous I rays, and with storied structure in the Ulmaceae than in the Moraceae.

The description of the anatomy of this family is in substantial agreement with the descriptions in the books and papers cited on page 13, as well as with the work of JANSSONIUS (**86**).

URTICACEAE

The Urticaceae are widely distributed in both temperate and tropical zones, but they are most abundantly distributed in the tropics. The family is made up largely of herbs, some shrubs, and (very rarely) trees. According to ENGLER and PRANTL (**61**) there are 41 genera and about 550 species in this family. Twelve genera and 30 species were examined in the present study. The genera investigated are *Urtica* (perennial or annual herbs) (2 spp.), *Gyrotaenia* (shrubs or trees) (fairly young material), *Urera* (shrubs or trees) (3 spp.) (fairly young); *Laportea* (perennial herbs, shrubs, or trees) (4 spp.) (fairly young), *Boehmeria* (small trees, shrubs, undershrubs or herbs) (5 spp.) (fairly young), *Neraudia* (shrubs)(2 spp.) (young), *Pipturus* (trees and shrubs) (3 spp.) (fairly young), *Touchardia* (shrubs) (young), *Debregeasia* (shrubs), *Villebrunea* (shrubs) (3 spp.), *Leucosyke* (trees or shrubs) (fairly young), and *Myriocarpa* (trees or shrubs) (4 spp.) (fairly young).

Only 3 species have growth rings.

The fibrous tracheary elements are either fiber-tracheids or libriform wood fibers; if fiber-tracheids, the borders of the pits are small. Two species have fiber-tracheids alone (both of these have septate fiber-tracheids, exclusively). Fourteen species have both fiber-tracheids and libriform wood fibers: 3 of these have non-septate fibrous elements; 4 have some septate fiber-tracheids and septate wood fibers along with the non-septate fiber-tracheids and libriform wood fibers; and 7 have all septate fiber-tracheids and septate wood fibers (fig. 21). Six species have libriform fibers: 4 of these have the non-septate type and 2 have the septate type. To summarize the fiber situation in another way: 7 species have non-septate fibers and 15 have septate fibers.

Vessel distribution is a combination of solitary pores and pore

multiples (fig. 22). In only 1 species are pore clusters found along with these other 2 types of vessel pattern. In any case the percentage of solitary pores is usually higher than the percentage of multiples. All the species are diffuse-porous. As for the vessel outline in transverse section, 1 species is angular, 10 species are angular and round (fig. 22), and 11 species are round. Most of the species (14) have thin vessel walls (fig. 22), 4 have thin and thick, and 4 have thick walls. The vessel diameters are very small to large (averages range 54 to 226 μ; mean of the family 125 μ). Tyloses are present in 18 species; 15 species with many and 3 with a few tyloses. All those with tyloses have the thin-walled type. All the vessel elements have simple perforation plates. The end walls of the vessels vary from 20° to 90°. Sixteen species have end walls at 90° (fig. 23); 9 of these have a few vessel members with end walls at 90° and 7 have many vessel members with end walls at 90°. Six species do not have any vessel elements with end walls at 90°. All the species have crowded, alternate intervascular pitting. The vessel elements are extremely short to medium sized (averages range 190 to 388 μ; mean of the family 284 μ). There are no spiral thickenings in the vessels.

As for ray type, 20 species have heterogeneous I rays and 4 species have heterogeneous IIA rays. Only 1 of these species with IIA rays has IIA rays in the strict sense. The others are nearly heterogeneous I rays for they have some upright cells in the multiseriate portion. The heterogeneous I rays are very odd (fig. 21) for they have upright cells in the multiseriate portion of the ray and yet the uniseriate wings are very short. Furthermore, there are but very few uniseriate rays and these are low; in fact, 2 species have no uniseriate rays. In width, the rays vary from 1 to 18 cells. However, all but 3 species are 1–9 cells wide. In depth, the uniseriate rays vary from 1 to 16 cells; the multiseriate rays from 4 to 350 cells.

Most often (16 spp.) the xylem parenchyma is vasicentric (fig. 22). At times (4 spp.) it is vasicentric and metatracheal; also vasicentric and aliform (1 sp.), or terminal, vasicentric, and confluent (1 sp.). There are some fusiform parenchyma cells in 9 species.

There is storied structure in 9 species (fig. 23). In 6 of these forms the seriation is marked; that is, xylem parenchyma strands, fusiform parenchyma cells, fibers, and vessel elements are storied—but not

the rays. In 3 of the species the seriation is not so marked, being present only in the xylem parenchyma cells. All the species have alternate vessel-parenchyma pitting. All forms have some unilateral compound pitting.

There are crystals in the ray cells of some (5) species. Two species have crystalliferous chambered parenchyma cells among the parenchyma strands.

Fourteen species in 5 genera (*Gyrotaenia*, *Urera*, *Laportea*, *Touchardia*, and *Myriocarpa*) have unlignified parenchyma patches in the xylem. These unlignified areas occurred in all the species of the above genera examined and 1 species of *Urtica*. They are made up of unlignified wood parenchyma strands, unlignified fusiform parenchyma cells, and unlignified ray cells. The areas may be in the form of islands (fig. 24), or of bands, or of combinations of both. They have no relation to the pores of the wood. In some species the wood parenchyma cells in the unlignified zones are very large. At first glance these unlignified regions resemble patches of included phloem, but closer examination fails to reveal any evidence of sieve plates on the cells.

The herbaceous members are of the "continuous" stele type. Internodal transverse sections demonstrate that the xylem is in the form of an uninterrupted ring. At the nodes the xylem ring is discontinuous in places, owing to the fact that the traces to the leaves are flanked, and at times subtended by parenchyma. It is interesting to note that some of the herbs have areas of unlignified parenchyma cells, as do the shrubs and trees.

On the whole the Urticaceae are higher anatomically than are the Moraceae. Both families have alternate intervascular pitting and simple perforation plates on the vessel members. The Urticaceae are higher, however, in that: they have a higher percentage of forms with septate fibers, they have more storied structure, they are largely herbaceous, the vessel elements are shorter, they have a higher percentage of forms with the end walls of vessel elements at 90°, and all the species have unilateral compound pits. Further, in the Urticaceae are forms with unlignified parenchyma areas. BAILEY (11) has indicated that such anomalous structures are indicative of highly specialized woods.

On the other hand, it would appear that the Urticaceae are more

primitive than the Moraceae, for in the Urticaceae there are many forms with angular, or angular to round, pores, the vessel diameter is slightly smaller, and the ray type is lower.

These last few inconsistencies can be explained by the fact that the Urticaceae are mostly shrubs and herbs, and as such they contain but one, or at most a few, growth layers; therefore they consist of "young" wood. With this essentially "young" xylem, one would expect to find that the vessel elements are small, the pores angular, and the rays heterogeneous. The fact that in the forms with heterogeneous I rays, the multiseriate rays have very short wings and that there are very few, or no, uniseriate rays would seem to indicate that these heterogeneous rays might become homogeneous in subsequent growth layers, as happens so often in trees. Further, the species with xylem obviously taken some distance from the pith have heterogeneous IIA rays. It will be recalled that the two highly specialized moraceous herbs, *Cannabis* and *Humulus*, also have heterogeneous I rays. In other words, the Urticaceae occupy the same relation to the Urticales as do the Cannaboideae to the Moraceae.

Rhoipteleaceae

The Rhoipteleaceae are a new family (**80**) which is placed in the Urticales by Wettstein (**158**) and by Engler and Diels (**60**). Only one species, *Rhoiptelea chiliantha* Diels and Hand. from Tonking and southwestern China, has been described. It is a tree.

Growth rings are present. All the fibrous tracheary elements are fiber-tracheids. In a transverse section there are many solitary pores, some pore multiples, and a few pore clusters (fig. 25). There is a tendency toward ring-porosity. The vessels are thin-walled and mostly angular to round. The vessels are very small to moderate sized, but mostly small (range 30 to 120 μ; mean 81 μ). There are many thin-walled tyloses. The perforation plates on the vessel elements are exclusively scalariform. The perforations are non-bordered and wide (4 to 30 μ). There are a few (3 to 8, mostly 3 to 5) bars on each plate. The vessel element end walls vary from 20° to 55°. The intervascular pitting is alternate. These pits are rather small. The vessel elements are medium sized to moderately long (range 640 to 950 μ; mean 794 μ).

The rays are heterogeneous I, bordering on heterogeneous IIA.

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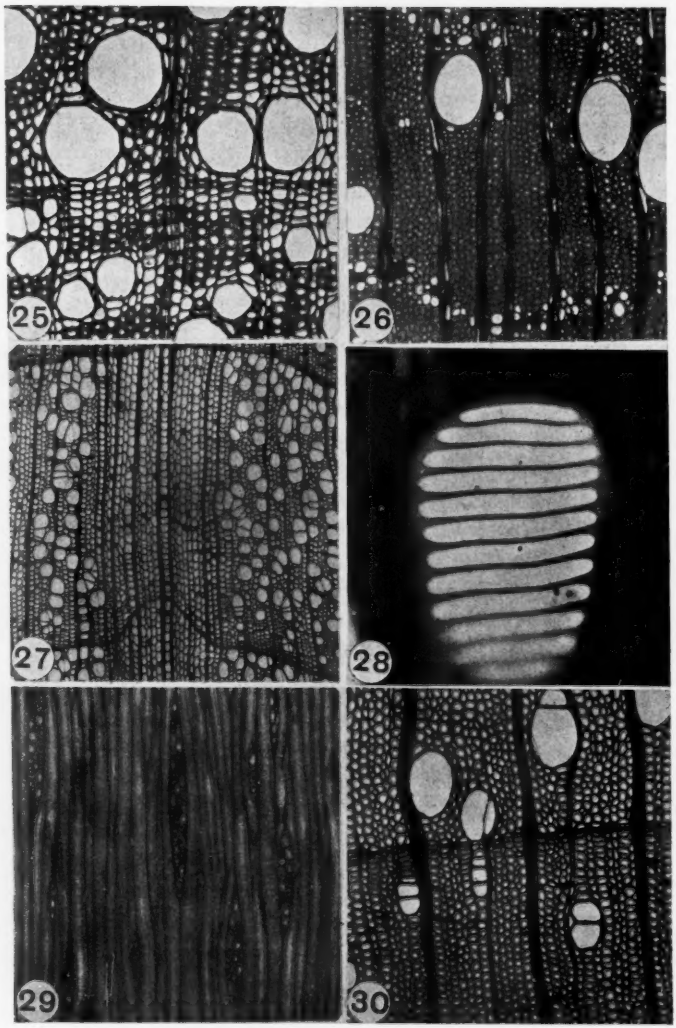


FIGS. 25–30.—Fig. 25, *Rhoiptelea chiliantha* Diels and Hand., Rhoipteleaceae; transverse section. ×90. Fig. 26, *Casuarina equisetifolia* L., Casuarinaceae; transverse section. ×80. Fig. 27, *Alnus japonica* Sieb. and Zucc., Betulaceae; transverse section. ×50. Fig. 28, *Alnus rhombifolia* Nutt., Betulaceae; scalariform perforation plate. ×450. Fig. 29, *Betula grossa* Sieb. and Zucc., Betulaceae; tangential section. ×100. Fig. 30, *Betula davurica* Pall., Betulaceae; transverse section. ×90.

Ray width is from 1 to 5 cells. In depth, the uniseriate rays vary from 2 to 25 cells and the multiseriate rays from 7 to 83 cells.

As for xylem parenchyma distribution, there is a single row of terminal parenchyma, in addition to ordinary vasicentric, aliform, and some confluent (fig. 25). The vessel parenchyma pitting is transitional, opposite, and alternate.

The fact that the perforation plates are exclusively scalariform and that the vessel elements are so long makes it obvious that this family does not belong in the Urticales. The combination of these and other primitive characters, such as the presence of fiber-tracheids, mostly angular vessels, oblique vessel element end walls, heterogeneous I rays, and transitional, opposite, and alternate vessel parenchyma pitting, indicates that this family belongs with some much more primitive order than the Urticales.

The anatomical description given here agrees for the most part with that of TANG (**137**). However, the writer cannot agree that there is any close resemblance between the ulmaceous genus *Aphananthe* and *Rhoiptelea*. The parenchyma distribution is the same in the two; but then the same combination of vasicentric and terminal parenchyma can be found in dozens of woods. The type of vessel element in the two genera is quite different, as TANG points out.

CASUARINACEAE

The Casuarinaceae are mainly an Australian group, but are also found on the Mascarene Islands, in southeast tropical Asia, on New Caledonia, and on some of the other islands of the Pacific Ocean. They are trees and shrubs. ENGLER and PRANTL (**61**) state that there are about 30 species in the single genus, *Casuarina*. Eleven species were examined in this study. Six of these species were rather small shrubs, and therefore the wood examined came from a region close to the pith.

There are growth rings in 2 species, traces of growth rings in 4, and none in 5 species.

The fibrous tracheary elements are all tracheids. The bordered pits on the tracheids are not large, but since they are as large as the small bordered pits on the vessel elements, the fibrous elements must be designated tracheids.

Most of the pores are solitary (fig. 26); there are a few pore multi-

ples, however. All the woods are diffuse-porous. The pores are angular in 4 species and angular to round in 7 species. The vessel walls are thin. The vessel diameters are extremely small to moderate sized, but mostly small and moderate sized (range 10 to 140 μ; mean 88 μ). Seven of the species have scalariform and simple perforation plates on the vessel members; 3 of these have 50 per cent of each class and 4 have mostly simple perforation plates. Three species have vestigial scalariform perforation plates along with the simple. In the 7 species with scalariform and simple perforation plates, all have non-bordered perforations. Six of these species have the intermediate number of bars and 1 species has a few bars. All 7 of these species have narrow perforations. The end walls on the vessel elements vary from 15° to 90°. In 2 species there are some vessel members with end walls at 90°. In all the species the intervascular pitting is transitional (some), opposite (most), and alternate (some). Vessel element length varies from moderately short to medium sized (range 270 to 570 μ; mean 412 μ). Spiral thickenings are present in some of the vessel members of 4 species.

The rays are heterogeneous IIB, bordering on homogeneous I at times. The rays are from 1 to about 50 cells in width. Aggregate rays are found in all 11 species; absent in old stems in 4 species and present in old stems in 7. Very wide or so-called "compound" rays are present in 6 species. So-called "diffuse" rays are found in 4 species. To summarize the ray situation in another way: in 4 species the aggregate and "compound" rays of the young stems pass over to the "diffuse" condition in the older stems and in 7 species there are aggregate and "compound" rays to both young and old axes. In depth, the uniseriates run from 1 to 18 cells and the ordinary multiseriates run from 5 to 40 cells. The aggregate and "compound" rays are hundreds of cells in height.

The xylem parenchyma is metatracheal in 5 species. Here the bands of parenchyma cells vary from 1 to 7 cells in width. In 3 species there is diffuse and metatracheal parenchyma (fig. 26). The bands are 1 to 4 cells wide. The vessel parenchyma pitting in all is transitional (some), opposite (most), and alternate (some).

Some of the species have chambered parenchyma cells with crystals.

It is apparent that the Casuarinaceae are rather highly specialized anatomically. The vessel elements are fairly short, some of the species have vessel members with end walls at 90°, the rays are almost homogeneous I, the parenchyma is metatracheal, and the intervascular pitting is mostly opposite with some alternate. Furthermore, the vessel elements are mostly simple in 4 species and simple with vestiges of the scalariform state in 3 other species. Anatomically the Casuarinaceae cannot be regarded as the most primitive, or among the most primitive, of the families of the angiosperms, as they have often been considered by certain phylogenists.

On the other hand, the Casuarinaceae are not so highly specialized anatomically as the Urticales. The former have tracheids, angular or angular to round vessels, some scalariform perforation plates, and opposite (mostly) intervascular pitting.

This anatomical description of the Casuarinaceae is in agreement with that of BOODLE and WORSDELL (**35**).

BETULACEAE

The Betulaceae are characteristic of the north temperate regions of both hemispheres. In the Old World, some species of *Alnus* occur as far south as Bengal, while in the New World 1 species of *Alnus* occurs as far south as Argentina. Species of *Ostrya* and *Carpinus* are found in Mexico. The family consists of trees and shrubs. There are 6 genera and some 80 species in the Betulaceae. The anatomical description which follows is based on a study of 6 genera and 46 species. The genera studied are *Ostryopsis* (shrubs), *Carpinus* (8 spp.), *Ostrya* (3 spp.), *Corylus* (4 spp.), *Betula* (20 spp.), and *Alnus* (10 spp.).

All the species except *Betula cylindrostachya* Wall. have growth rings.

There are some tracheids along with the fiber-tracheids in *Alnus* and *Betula*. In *Carpinus*, *Ostrya*, and *Corylus* some of the fiber-tracheids are quite close to tracheids. *Ostryopsis* has fiber-tracheids.

In 5 genera there are solitary pores, pore multiples, and pore clusters, and in *Ostrya* there are solitary pores and pore multiples. All the genera are diffuse-porous. The vessels are angular in 43

species and angular to round in 3. All the forms have thin-walled vessels. Vessel diameters are extremely small to moderate sized, but mostly very small (fig. 27) and small (averages range 32 to 66 μ, mean 48 μ). Tyloses are present in *Ostrya*. These tyloses are thin-walled and many. The perforation plates are exclusively scalariform in 37 species (fig. 28). In 9 species (most *Carpinus* species and all the species of *Ostrya*) the plates are mostly simple with some vestigial scalariform plates. By far, most of the scalariform plates are non-bordered (fig. 28). In the 5 genera with scalariform perforation plates, 2 have plates with many bars and 3 have plates with the intermediate number of bars (fig. 28). Two genera have narrow perforations and 3 have wide. Vessel element end walls vary from 15° to 50°. Intervascular pitting is alternate in 5 genera and transitional (some), opposite (most), and alternate (some) in *Alnus*. Vessel element length is medium sized to moderately long, mostly medium sized (averages range 421 to 743 μ; mean of the family 608 μ). There are spiral thickenings in the vessel elements of 11 species; absent in 35. Four of the species with spiral thickenings have scalariform perforation plates exclusively (*Ostryopsis*, 1 species of *Carpinus*, and 2 species of *Corylus*). Seven of the species with spiral thickenings have simple and vestigial scalariform perforation plates (4 species of *Carpinus*, all 3 species of *Ostrya*).

There are heterogeneous IIB rays in *Ostryopsis* and *Corylus*, but even here the rays are almost homogeneous I. The rays are homogeneous I (fig. 29) in 4 genera. In 2 of the latter genera the rays border on heterogeneous IIB. In width, the rays vary from 1 to 4 cells, except in *Alnus rhombifolia* Nutt. where the rays are very wide (up to 25 cells) or so-called "compound." In depth, the uniseriate rays are from 1 to 50 cells high and the multiseriate rays from 3 to 80 cells, except the species with very wide rays, for here the wide rays are many hundreds of cells high. Aggregate rays (fig. 27) are present in 23 species.

The xylem parenchyma is diffuse, terminal (bands 1 to 3 cells in width), and metatracheal (in the form of inconspicuous, meandering, uniseriate rows) in 9 species. Nine species have terminal (1 to 2 cells in width) and metatracheal parenchyma (in the form of inconspicuous, meandering, uniseriate rows). Nine species have diffuse and

terminal (1 to 3 cells in width) (fig. 30). Ten species have diffuse, terminal (1 cell in width), and vasicentric (just a few cells about the vessels, not forming sheaths). One species, *Betula cylindrostachya* Wall., has diffuse and metatracheal (bands 1–3 cells wide) parenchyma. Vessel-parenchyma pitting is opposite and alternate in *Alnus* and alternate in the other 5 genera.

Crystals are found in the ray cells of 3 genera. Pith flecks are present in some (8) species.

Table 4 is a summary of the anatomical data concerning the tribes Betuleae and Coryleae. It is obvious that the Betuleae are more primitive than the Coryleae. As far as three characters are concerned, however, it would seem as though the Betuleae are higher than the Coryleae: Three species of the Betuleae have angular to round vessels. The mean of the vessel diameter averages in the Betuleae is slightly higher than the mean in the Coryleae. This can be explained by the fact that the measurements on *Ostryopsis* and *Corylus* were made on young material. In the Coryleae, 2 species have heterogeneous IIB rays. However, these rays are almost homogeneous I rays; further they also were found in young material.

The Betulaceae are on a lower plane of anatomical specialization than the Casuarinaceae. The Betulaceae have a much greater range of variation than the Casuarinaceae. Some of the genera are as high as *Casuarina*, therefore, whereas most of the genera are considerably lower. The Betulaceae are lower than the Casuarinaceae in the following ways: most of the vessels are angular, the vessel diameter is less, most species have perforation plates which are exclusively scalariform, the vessel length is greater, the parenchyma distribution is diffuse and vasicentric in 50 per cent of the species, and there are no species with vessel element end walls at 90°. On the other hand, the Casuarinaceae seem more primitive in certain ways, for they have tracheids, a lower type of intervascular pitting, and heterogeneous IIB rays.

The anatomical description here given agrees in the main with the accounts by Bailey (**5, 6, 7**), Bailey and Sinnott (**16**), Hoar (**81**), Wetmore (**154, 155**), and Abbe (**1**). On the basis of secondary xylem anatomy, Abbe found that *Betula* and *Alnus* are most primi-

tive, *Corylus* and *Ostryopsis* less so, and *Carpinus* and *Ostrya* the least primitive. The results obtained in the present investigation support ABBE's conclusion.

TABLE 4

DATA ON THE TRIBES OF THE BETULACEAE

	BETULEAE (2 GEN., 30 SPP.) No. OF GENERA OR SPECIES	CORYLEAE (4 GEN., 16 SPP.) No. OF GENERA OR SPECIES	PERCENTAGE	
			BETULEAE	CORYLEAE
Tracheids (some)	2	0	100	0
Fiber-tracheids (all)	0	4	0	100
Angular vessels	27	16	90	100
Angular to round vessels	3	0	10	0
Range of vessel diameter averages	44–54μ	32–66μ	.....	.....
Mean of vessel diameter averages	49μ	47μ	.....	.....
Exclusively scalariform perforation plates	30	7	100	43.7
Simple and vestiges of scalariform perforation plates	0	9	0	56.3
Many bars on plates	2	0	100	0
Intermediate bars on plates	0	3	0	100
Transitional, opposite, and alternate intervascular pitting	1	0	50	0
Alternate intervascular pitting	1	4	50	100
End walls of vessel elements	15°–35°	15°–50°	.....	.....
Range of vessel element length averages	717–743μ	421–651μ	.....	.....
Mean of vessel element length averages	730μ	547μ	.....	.....
Spiral thickenings absent	2	5	100	31.3
Spiral thickenings present	0	11	0	68.7
Heterogeneous IIB rays	0	2	0	50
Homogeneous I rays	2	2	100	50
Ray width	1–4*	1–4	.....	.....
Uniseriate ray depth	1–28	1–50	.....	.....
Multiseriate ray depth	5–29*	3–80	.....	.....
Aggregate rays absent	20	3	66.3	18.7
Aggregate rays present	10	13	33.3	81.3
Diffuse and vasicentric parenchyma	19	0	85.5	0
Metatracheal parenchyma	3	16	14.5	100
Opposite and alternate vessel-parenchyma pitting	1	0	50	0
Alternate vessel-parenchyma pitting	1	4	50	100

* Disregarding very wide rays of *Alnus rhombifolia* Nutt.

FAGACEAE

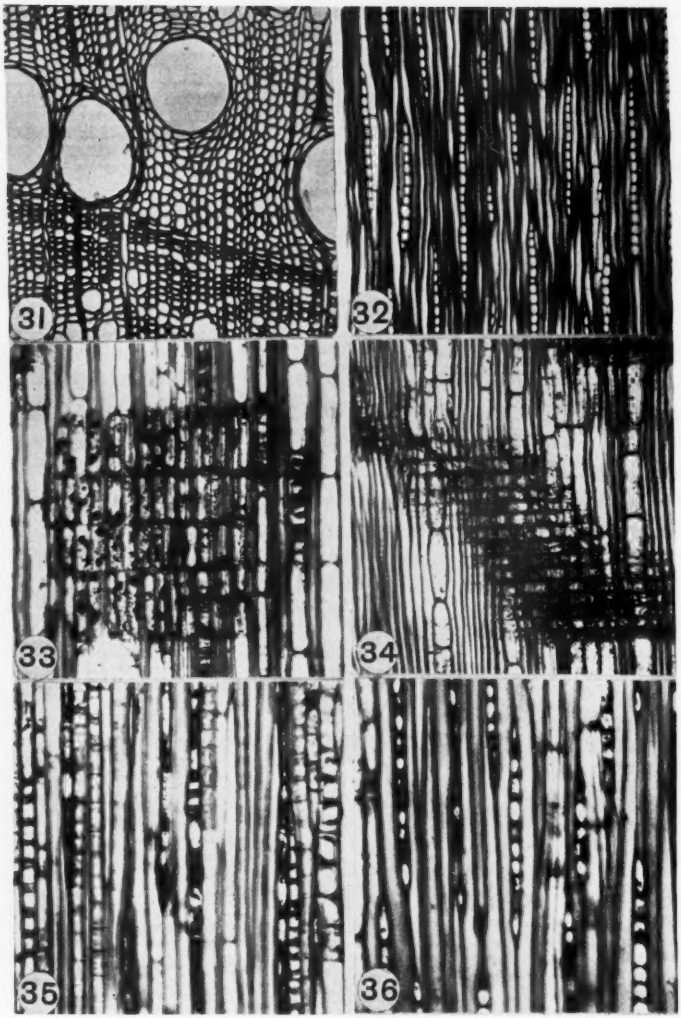
The Fagaceae are found in temperate and tropical regions, but they are absent from tropical and South Africa. The plants are generally trees; there are some shrubs, however. The family consists of

7 genera and about 350 species. The present study is based on an examination of 6 genera and 84 species. The genera include *Fagus* (5 spp.), *Castanea* (4 spp.), *Castanopsis* (4 spp.), *Pasania*, *Pasaniopsis*, and *Quercus* (69 spp.).

All have growth rings except 3 species of *Quercus* (*Q. engelmanni* Greene, *Q. gilva* Blume, and *Q. bennettii* Miq.).

One genus has tracheids and 5 genera have tracheids and fiber-tracheids. The tracheids in the latter group are vasicentric.

The vessels are mostly solitary with some multiples and clusters. Twenty-six species are diffuse-porous and 57 are ring-porous (fig. 31). The vessels are angular in 4 species, angular to round in 15 (fig. 31), and round in 4. The vessel walls are thin in 19 species, thin to thick in 5, and thick in 4. Vessel diameters range all the way from extremely small to very large, but are mostly small to moderate sized (averages range 46 to 120 μ; mean of the family 75 μ). Tyloses are absent in 1 genus and present in the other 5 genera. The tyloses are thin-walled and many. In one species of oak, *Quercus engelmanni*, the tyloses are sclerotic. The perforation plates on the vessel elements are scalariform and simple in 16 species, of which 2 species have mostly scalariform and 14 have mostly simple. Two species have simple and vestigial scalariform perforation plates. Two species have exclusively simple perforation plates. These perforation plate figures include but 7 species of *Quercus* in order not to distort the picture of the family as a whole by the vessel condition in the many species of *Quercus*. The various species of *Quercus* exhibit considerable variation as regards the type of perforation plate. Some species have simple and scalariform (mostly simple) plates, others have simple and vestigial scalariform, and still others have simple plates exclusively. Most of the forms in the family with scalariform plates have non-bordered apertures; 1 species has the apertures bordered to the middle and 1 species has them bordered at the ends. In 1 species there are many bars, intermediate in number in 2, and a few in 7. The apertures or perforations are wide in all except 1 of the species. The end walls of the vessel members vary from 20° to 90°. *Quercus* has some vessel elements with end walls at 90°; the other 5 genera have no vessel elements with end walls at 90°. The intervascular pitting is transitional and opposite in 1 genus, opposite in



FIGS. 31–36.—Fig. 31, *Pasania sieboldii* Makino, Fagaceae; transverse section. ×80. Fig. 32, *Castanea pumila* Mill., Fagaceae; tangential section. ×80. Fig. 33, *Castanopsis argentea* A. DC., Fagaceae; radial section of region close to pith, showing heterogeneous ray. ×120. Fig. 34, same, radial section of region some distance from pith, showing that rays have become quite homogeneous. ×100. Fig. 35, same, tangential section of region close to pith, showing heterogeneous rays. ×150. Fig. 36, same, tangential section of region some distance from pith, showing that rays have become quite homogeneous. ×150.

4, and opposite and alternate in 1. The vessel lengths vary from extremely short to moderately long, but mostly moderately short and medium sized (averages range 290 to 575 μ; mean of the family 473 μ). It is odd that there are no spiral thickenings in the vessel elements and yet this family has many temperate, ring-porous woods.

The rays are heterogeneous IIB in 1 genus, homogeneous I in 1, and homogeneous III (fig. 32) in 4 genera. In 2 of the genera with the latter type of ray, the rays are rarely 2 cells wide. In the other 2 genera the rays are slightly heterogeneous in places. Figure 33 is a photomicrograph of a radial section of the xylem of *Castanopsis argentea* A. DC. taken from a region close to the pith. The rays are strikingly heterogeneous in this zone. Figure 34 is a similar view from the same species, but here the xylem pictured is from a region which is a considerable distance from the pith. It is obvious that the rays are homogeneous. In other words, close to the pith the rays are heterogeneous while some distance out they are homogeneous. This heterogeneity of the rays in youthful regions is of great importance in interpreting young material, small shrubs, and herbs. Figure 35 is a tangential view of the rays in a "young" region, and figure 36 is the same in a "mature" region.

The rays vary from 1 to 40 cells in width. In all except *Fagus* the rays are of two distinct types without any intermediate forms; that is, uniseriate rays and very wide, or so-called "compound rays." The uniseriate rays are from 1 to 48 cells high. The multiseriate rays run from 7 cells to thousands of cells high. Very wide rays, or "compound rays," are present in *Fagus*, in *Quercus*, and sometimes in *Castanea*. The other 3 genera have uniseriate rays. *Quercus palmeri* Engelm. and *Q. lappacea* Roxb. have no "compound" rays. The former does have aggregate rays; the latter has only uniseriate rays. Aggregate rays are present sometimes in the young wood of *Castanea* and sometimes in the young wood of *Quercus* when they are absent in the mature wood. Seventeen of the 69 species of *Quercus* examined have aggregate rays in the mature wood. The form with the sclerotic tyloses, *Q. engelmanni* Greene, has sclerotic ray cells also.

The xylem parenchyma is diffuse and metatracheal (fig. 31) in

most of the species. The metatracheal parenchyma is in the form of uniseriate rows usually; in 2 species these bands are 1 to 3 cells in width. In 2 species the xylem parenchyma is diffuse. Vessel-parenchyma is transitional and opposite in 1 genus, opposite in 4, and opposite to alternate in 1.

Crystals are found in the ray cells of 2 genera. Crystalliferous chambered parenchyma cells (fig. 35) among the parenchyma strands and among the ray cells are present in some species of *Quercus* and *Castanopsis*.

The Fagaceae are more highly specialized anatomically than are the Betulaceae, for in the Fagaceae the vessels are mostly angular to round, or round; the vessel walls are often thin to thick, or thick; the vessel diameter is greater; the perforation plates of the vessel elements are of a higher type; some species have vessel member end walls at 90°; the vessel element length is less; the ray type is higher; and the woods are often ring-porous. On the other hand, the Betulaceae seem more highly evolved as far as two characters are concerned. The intervascular pitting is mostly alternate, and most of the genera have fiber-tracheids exclusively, whereas in the Fagaceae there are always tracheids present. However, these tracheids in the Fagaceae are vasicentric and therefore highly specialized structures occurring about the large vessels of the ring-porous woods and the vessels of diffuse-porous woods.

The preceding anatomical description of the Fagaceae agrees substantially with the account by BAILEY (**5**) and with various other descriptions (**56, 6, 7, 16, 154, 155**) of ray conditions in the family.

JUGLANDACEAE

The Juglandaceae inhabit the warmer parts of the north temperate zone, extending into tropical eastern Asia to China and Japan. The plants of this family are trees placed in 7 genera and some 37 species. The anatomical description given here is based on a study of 5 genera and 18 species. The genera investigated are *Engelhardtia* (2 spp.), *Pterocarya*, *Juglans* (7 spp.), *Carya* (7 spp.), and *Alfaroa*.

Growth rings are present in 16 species; there is a trace in 1 and no growth rings in 1 species.

There are tracheids and fiber-tracheids in 1 species. Seventeen

species have fiber-tracheids exclusively; 6 of the latter have fiber-tracheids which are nearly libriform wood fibers.

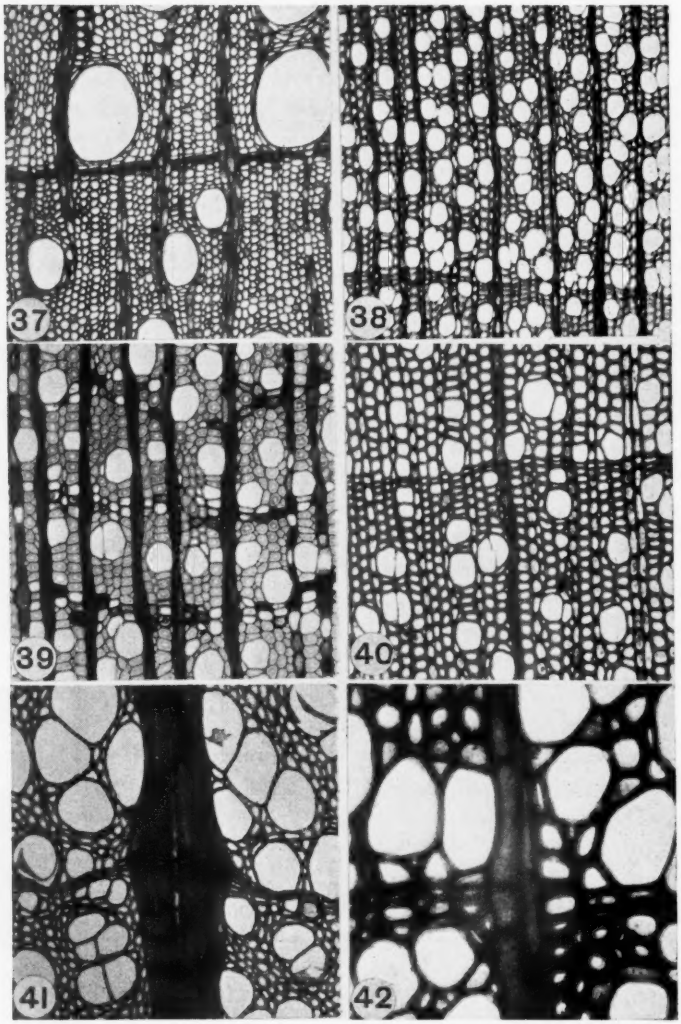
The vessels are solitary, in multiples, and in clusters in 3 genera. In 2 genera the vessels are solitary and in multiples. Eight species are diffuse-porous, 4 are semiring-porous, and 6 are ring-porous (fig. 37). The vessels are angular in 1 species and angular to round in 17 species (fig. 37). The walls of the pores are thin in 11 (fig. 37) and thick in 7 species. The vessel diameters vary from very small to rather large, but mostly small and moderate sized (averages range 68 to 154 μ; mean of the family 119 μ). Tyloses are present in 16 of the 18 species. In 2 species there are only a few; in 14 species there are many. In any case the tyloses always have thin walls. The perforation plates on the vessel members are scalariform and simple in 2 species, simple and vestigial scalariform in 1, and simple in 15 species. The 2 species with simple and scalariform perforation plates have wide, non-bordered perforations and there are only a few bars to each plate. Vessel element end walls vary from 20° to 75°. Intervascular pitting is transitional (some), opposite, and alternate in 1 species and alternate in 17 species. Vessel member length varies from very short to very long, but is mostly medium sized (averages range 368 to 718 μ; mean of the family 528 μ).

The rays are heterogeneous I in 1 species, heterogeneous IIA in 4, and heterogeneous IIB in 13 species (2 of the latter species have rays close to IIA). The rays are 1 to 6 cells in width. The uniseriates are 1 to 40 cells in depth and the multiseriate rays are 4 to 75 cells high.

In most species (7) the xylem parenchyma is terminal and confluent. In 4 species it is terminal, metatracheal, vasicentric, and confluent. In 2 species it is terminal, metatracheal, and vasicentric. In 1 species it is diffuse, metatracheal, and vasicentric and in another it is terminal, metatracheal, and confluent. Vessel-parenchyma pitting is transitional, opposite, and alternate in 1 species and alternate in 17. Unilateral compound pitting is found in 2 species.

Crystals are present in the ray cells of certain species. Crystalliferous chambered parenchyma cells among the wood parenchyma strands are found in 3 species. There are crystals in the swollen xylem parenchyma cells in all the species of *Carya*.

All in all, the Juglandaceae are more highly specialized ana-



FIGS. 37–42.—Fig. 37, *Juglans californica* S. Wats., Juglandaceae; transverse section. ×55. Fig. 38, *Hamamelis virginiana* L., Hamamelidaceae; transverse section. ×80. Fig. 39, *Distylium racemosum* Sieb. and Zucc., Hamamelidaceae; transverse section. ×90. Fig. 40. *Stachyurus praecox* Sieb. and Zucc., Stachyuraceae; transverse section. ×110. Fig. 41, *Platanus occidentalis* L., Platanaceae; transverse section. ×90. Fig. 42, *Eucommia ulmoides* Oliver, Eucommiaceae; transverse section. ×380.

tomically than are the Fagaceae. This is evident when it is remembered that in the former the fibrous tracheary elements are of a higher type, the vessels are thick-walled in many species, the vessel diameter is greater, most of the species have simple perforation plates, and most of the species have alternate intervascular pitting. Yet in the Juglandaceae the vessel elements are longer, the ray type is lower, and there are no species with round vessels exclusively. The Juglandaceae are also on a higher plane, anatomically speaking, than are the Casuarinaceae. However, in the Casuarinaceae there are some species with vessel end walls at 90°. Further, the vessel elements are shorter and the ray type higher than in the Juglandaceae.

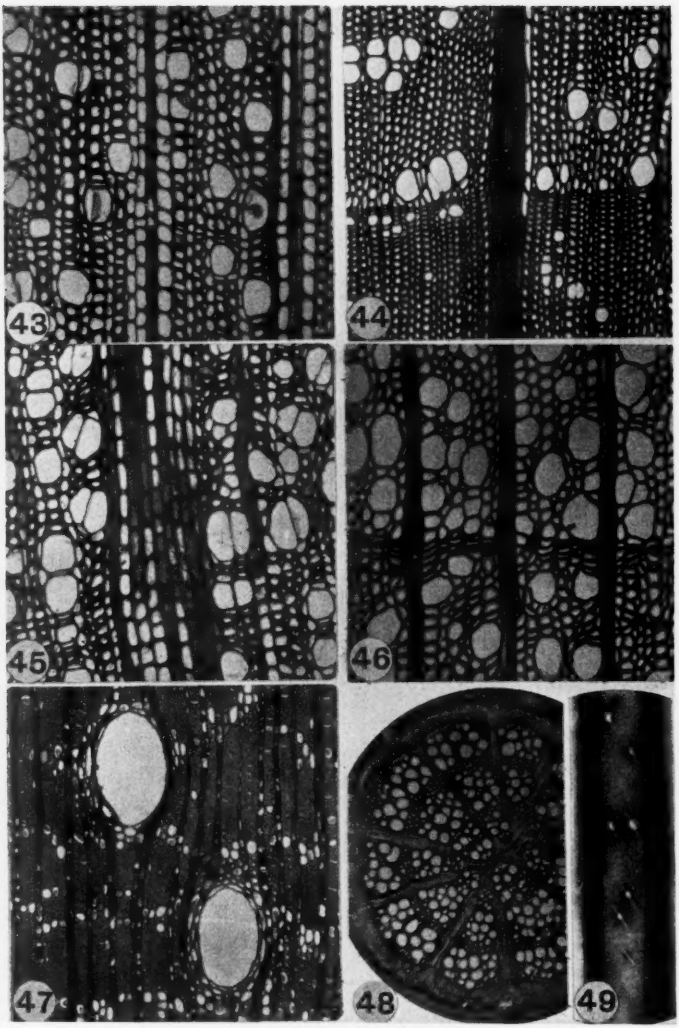
The anatomical description of the family given here is in agreement with that of KRIBS (**100**).

HAMAMELIDACEAE

The Hamamelidaceae are a family of trees and shrubs occurring in subtropical and temperate climates, especially in Asia and eastern North America. There are a few in Madagascar and South Africa. ENGLER and PRANTL (**62**) divide the family into 23 genera and about 94 species. The present investigation is based on a study of 18 genera and 30 species. The genera examined anatomically are *Disanthus* (very young), *Hamamelis* (2 spp.), *Loropetalum*, *Trichocladus*, *Maingaya* (very young), *Eustigma* (very young), *Corylopsis* (4 spp.), *Fortunearia* (very young), *Parrotia*, *Parrotiopsis* (very young), *Fothergilla* (young), *Distylium* (2 spp.), *Sycopsis* (3 spp.), *Sinowilsonia* (very young), *Rhodoleia* (2 spp.) (very young), *Bucklandia*, *Liquidambar* (3 spp.), and *Altingia* (3 spp.).

Growth rings are absent in but 3 species. Vertical intercellular canals, or gum ducts, of the traumatic or gummosis type (as defined by RECORD, **119**) are found in 3 species (in *Liquidambar styraciflua* L., but not in the other 2 species; and in *Altingia chinensis* (Oliver) Benth. and *A. obovata* Merr. and Chun., but not in *A. excelsa* Nor.).

The fibrous tracheary elements in 21 species are all tracheids, and tracheids and some fiber-tracheids in 1 species. That these are true tracheids in the BAILEY sense (**13**) is clear when the pits on the

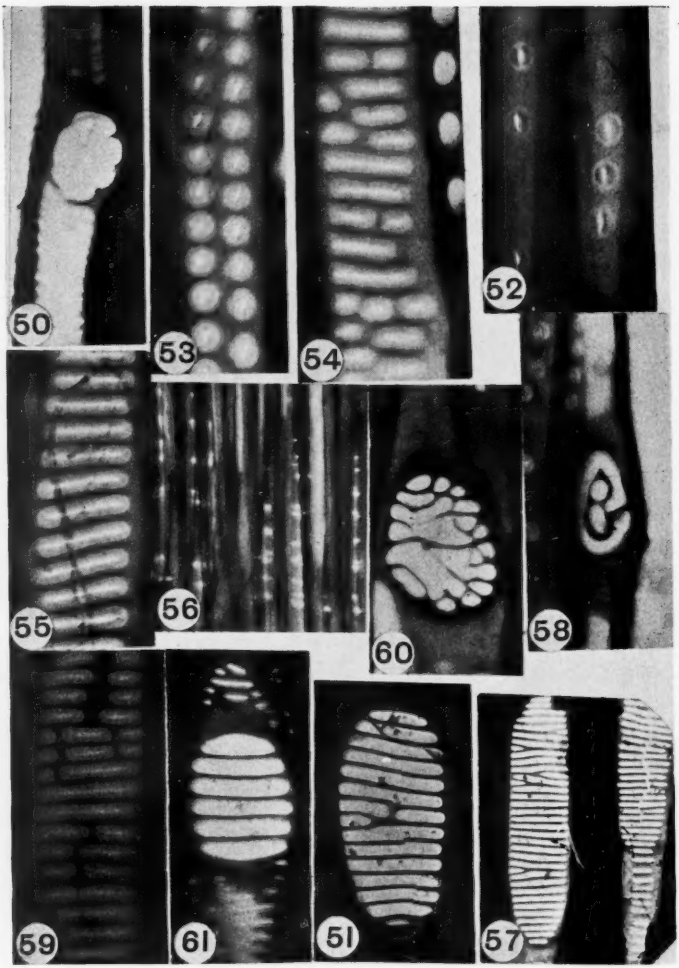


FIGS. 43–49.—Fig. 43, *Hydrangea quercifolia* Bartram, Hydrangeaceae; transverse section. ×100. Fig. 44, *Ribes viscosissimum* Pursh., Grossulariaceae; transverse section. ×90. Fig. 45, *Carpodetus serratus* Forst., Escalloniaceae; transverse section. ×90. Fig. 46, *Pyrus americana* DC., Rosaceae; transverse section. ×120. Fig. 47, *Parinarium gilletii* DeWild., Rosaceae; transverse section. ×50. Fig. 48, *Aristolochia tomentosa* Sims., Aristolochiaceae; transverse section. ×13. Fig. 49, *Planera aquatica* J. F. Gmel., Ulmaceae; fiber-tracheid bearing small bordered pits with extended apertures. ×700.

fibrous elements are compared with those on the vessel members (*cf.* figs. 52, 53).

Vessel distribution in transverse section in all the species is mostly solitary with but a very few multiples (figs. 38, 39). All the species are diffuse-porous (fig. 38). In all, the vessels are angular and thin-walled (figs. 38, 39). Vessel diameters are usually extremely small (fig. 38) and very small, rarely small (averages range 15 to 48 μ; mean of the family 32 μ). Thin-walled tyloses are found in 4 species. These are few (1 sp.) or many (3 spp.). In all the species the perforation plates on the vessel elements are exclusively scalariform (figs. 51, 57). In 7 species the perforations in the plates are bordered at the ends and in 16 species these apertures are non-bordered (figs. 51, 57). These figures are based on the condition of the majority of the vessels in a species, for in a given species some of the perforation plates, or parts of plates, may vary all the way from completely bordered to non-bordered. The perforation plates have many bars (fig. 57) in 14 species and the intermediate number of bars (fig. 51) in 9 species. The apertures on the plates are narrow (figs. 51, 57) in 19 species and wide in 4. In all the species the vessel end walls are very oblique, varying from 5° to 40°. The intervascular pitting is scalariform (fig. 55), transitional (fig. 54), and opposite (fig. 53) in 18 species. In this group of 18 species the pitting is mostly transitional and opposite, or mostly opposite. The intervascular pitting in 3 species is transitional and opposite; and in 1 other species it is opposite. Vessel element length is medium sized to extremely long, but is mostly moderately long and very long (averages range 760 to 1598 μ; mean of the family 1089 μ). Spiral thickenings are present in the tips or "ligules" of vessel elements in 9 species, in all parts of the vessel elements in 1 species, and are absent from 20 species.

The rays are heterogeneous I in 9 species, heterogeneous IIA in 8 species, and heterogeneous III in 5 species. In the last group, with uniseriate, heterogeneous III rays, 3 of the species show a tendency to form multiseriate rays and the other 2 species are represented only by young material. Consequently, in older material this group of 5 species with heterogeneous III rays may be found to be heterogeneous I, or IIA. The rays of the family vary from 1 to 5 cells in width. The uniseriate rays are from 1 to 75 cells high and the multiseriates from 3 to 60 cells.



FIGS. 50–61.—Fig. 50, *Ulmus crassifolia* Nutt., Ulmaceae; vestigial scalariform perforation plate and spiral thickenings in vessel members. ×480. Fig. 51, *Hamamelis virginiana* L., Hamamelidaceae; scalariform perforation plate. ×350. Fig. 52, *Liquidambar styraciflua* L., Hamamelidaceae; tracheids with large bordered pits (*cf.* size of these pits with those on vessel element in fig. 53). ×500. Fig. 53, same, opposite intervascular pitting on vessel element. At the top the pitting appears alternate, owing to a slight twist in the element. ×500. Fig. 54, same, transitional intervascular pitting on vessel member. ×500. Fig. 55, same, scalariform intervascular pitting on vessel element. ×500. Fig. 56, *Trophis racemosa* Urb., Moraceae; simple pits on libriform wood fibers. ×510. Fig. 57, *Corylopsis wilsoni* Hemsl., Hamamelidaceae; scalariform perforation plate. ×220. Fig. 58, *Pyrus americana* DC., Rosaceae; perforation plate with modification of reticulate arrangement. ×520. Fig. 59, *Brunellia comocladifolia* Humb. and Bonpl., Brunelliaceae; transitional intervascular pitting on vessel element. ×400. Fig. 60, *Pyrus americana* DC., Rosaceae; reticulate perforation plate. ×450. Fig. 61, *Ribes viscosissimum* Pursh., Grossulariaceae; scalariform perforation plate. ×430.

Xylem parenchyma is diffuse (fig. 38) in 13 species, diffuse and terminal in 1, diffuse and metatracheal (fig. 39) in 5, and metatracheal in 2. One species has no xylem parenchyma, but this condition may be attributed to the youth of the specimen. Vessel-parenchyma pitting is scalariform, transitional, and opposite in 18 species, transitional and opposite in 3, and opposite in 1.

Crystalliferous chambered parenchyma cells among the wood parenchyma strands are present in 2 species. The same types of cells among the upright cells of the rays are present in 3 different species. In 2 additional species there are crystals in the ray cells.

Anatomically the Hamamelidaceae are the most primitive of all the families considered thus far. All but 1 species have tracheids exclusively; the vessels are mostly solitary; the woods are diffuse-porous; the vessels are angular, thin-walled, and extremely small; the perforation plates are exclusively scalariform; the end walls of the vessel members are very oblique; the intervascular pitting is scalariform, transitional, and opposite; the vessel elements are very long; the rays are usually heterogeneous I, or IIA; and the xylem parenchyma is diffuse in most species.

The secondary xylem of this family is very close in all anatomical details to certain groups in the Magnoliales as described by McLaughlin (**109**) (the writer has also examined most of the genera of this order). The Magnoliales exhibit a considerable range of anatomical structures, yet it may be said that on the whole the least specialized members of that order are more primitive anatomically than are the Hamamelidaceae. Four genera of the Magnoliales have no vessels (**143, 17, 109**). Most of the species with scalariform perforations have apertures which are bordered at the ends, or bordered to the middle, or completely bordered. Further, most of the pitting in the Magnoliales is scalariform and transitional. The fibrous tracheary elements are exceptionally long in certain species (**18**). Some of the species have scalariform pits on the tracheids in the secondary xylem.

Stachyuraceae

The Stachyuraceae are a small family of shrubs or small trees found in eastern Asia. The family consists of 1 genus and 2 species. The present study is based on a piece of young wood of *Stachyurus praecox* Sieb. and Zucc.

Growth rings are present. All the fibrous tracheary elements are tracheids. Some of the tracheids have spiral thickenings.

Most of the pores are solitary; some are in multiples (fig. 40). The wood is diffuse-porous. The vessels are angular and thin-walled (fig. 40). The pores are extremely small to very small, but mostly very small (range 20 to 42 μ; mean 34 μ) (fig. 40). The perforation plates on all the vessel elements are scalariform. Most of the perforations are bordered at the ends; some are bordered to the middle. There are many bars (about 36) on the plates and the apertures are narrow. The vessel element end walls are very oblique (7° to 30°). Intervascular pitting is mostly opposite, rarely transitional. The pits on the vessels are small. Vessel element lengths are moderately long to very long, mostly very long (range 1110 to 1300 μ; average 1225 μ). There are spiral thickenings in the vessel members.

The rays are heterogeneous I and from 1 to 4 cells in width. The uniseriate rays vary from 2 to 33 cells in depth and the multiseriates from 6 to 65 cells.

The xylem parenchyma distribution is diffuse (fig. 40). Vessel-parenchyma pitting is mostly opposite, rarely transitional.

The anatomy of the Stachyuraceae is very similar to that of the Hamamelidaceae. However, WETTSTEIN (**158**) and ENGLER and PRANTL (**62**) place the former in the Parietales in the vicinity of the Flacourtiaceae. It is obvious that mature wood and the xylem of the other species of the Stachyuraceae should be studied before a decision can be made as to which group it is more likely to be related. Judging from the descriptions of the anatomy of the Flacourtiaceae (**147, 148**), the Stachyuraceae do not belong very close to the former. However, further study may show that the Stachyuraceae are related to the Canellaceae,[5] or to some other family of the Parietales.

MYROTHAMNACEAE

The Myrothamnaceae are a family of small shrubs occurring in Africa and Madagascar. There is 1 genus and 2 species. A specimen

[5] VESTAL (**148**) suggests that the Canellaceae be taken out of the Parietales and placed near the Myristicaceae, as WETTSTEIN and BESSEY have done.

of *Myrothamnus flabellifolia* Welwit., containing about 15 growth rings, was studied in this investigation.

All the fibrous tracheary elements are tracheids. Most of the vessels are solitary; some are in multiples. The wood is diffuse-porous. The vessels are thin-walled and angular. The pores are extremely small to small, mostly very small (range 18 to 52 μ; mean 35 μ). All the perforation plates are scalariform. There are many bars (about 45) and the perforations are narrow. The vessel member end walls are very oblique (5° to 35°). The intervascular pitting is transitional and opposite. The pits on the vessel elements are very small. Vessel element length is moderately long (range 850 to 960 μ; mean 905 μ).

The rays are heterogeneous III, and therefore are all uniseriate. In depth, the rays vary from 1 to 25 cells.

No xylem parenchyma was observed. Vessel-parenchyma pitting is transitional and opposite.

Anatomically the Myrothamnaceae resemble the Hamamelidaceae very closely.

Buxaceae

The Buxaceae are generally shrubs, trees, or rarely herbs. The 7 genera and approximately 30 species have a scattered distribution in temperate and subtropical regions. The present study is largely based on 3 species of *Buxus*, although *Pachysandra terminalis* Sieb. and Zucc. was also examined.

Growth rings are present in 2 species of *Buxus*. The fibrous tracheary elements are all tracheids.

The pores are mostly solitary with a few pore multiples. The wood is diffuse-porous. The vessels are angular and thin-walled. The vessel diameter is extremely small (range 12 to 26 μ; mean 20 μ). The vessel elements have scalariform and simple perforation plates, but the simple plates are rare. The plates have an intermediate number of bars and are narrow and non-bordered. The end walls of the vessel elements vary from 10° to 50°. Intervascular pitting is mostly opposite, with some alternate. The pits on the vessels are very small. Vessel element length is medium sized to moderately long, mostly medium sized (range 540 to 990 μ; mean 711 μ).

The rays are heterogeneous IIA and from 1 to 3 cells in width.

The uniseriate rays vary from 1 to 17 cells high and the multiseriate rays from 5 to 25 cells high.

Xylem parenchyma distribution is diffuse. Vessel-parenchyma pitting is opposite and alternate.

ENGLER and DIELS (**60**) place this family in the Sapindales, while WETTSTEIN (**158**) puts it in the Tricoccae near the Euphorbiaceae. Since the woods of these two orders were not studied in this investigation, and since the study of the Buxaceae was based on such a poor representation of the family, no final deposition of the family is attempted at this time. However, the Buxaceae might very well be considered as being very close to the Hamamelidaceae.

PLATANACEAE

The Platanaceae are a family of large trees occurring in the eastern Mediterranean region to the Himalayas and in North America from Mexico to Canada. The single genus, *Platanus*, is divided into 6 species. The present study is based on an examination of the xylem of 3 species.

Growth rings are present in all. The fibrous tracheary elements are all tracheids.

The vessels are mostly solitary with some pore multiples. The wood is diffuse-porous. The vessels are thin-walled and angular (fig. 41) with a slight tendency toward rotundity in some vessels. The vessel diameters are very small to small, mostly small (range 46 to 94 μ; mean 74 μ). Tyloses, present in all the species, are thin-walled and many. The perforation plates on the vessel members are scalariform and simple. On most of the plates the wide apertures are bordered at the ends and the bars are many. The vessel element end walls vary from 15° to 55°. Intervascular pitting is mostly opposite, with some alternate and rarely transitional. Vessel element length is medium sized (range 430 to 640 μ; average 543 μ).

The rays are homogeneous I. They are from 1 to 15 cells in width. The uniseriates vary from 1 to 19 cells in depth and the multiseriates from 9 to several hundred cells. The rays are very wide, or so-called "compound," in all 3 species.

The xylem parenchyma is usually metatracheal, forming loose uniseriate lines (fig. 41). The parenchyma is rarely diffuse. Vessel-

parenchyma pitting is opposite and alternate, or less often transitional.

The anatomy of the Platanaceae resembles that of the Hamamelidaceae in many ways, yet the former is on a higher plane of anatomical specialization than is the latter. In the Platanaceae there is a tendency toward round pores, the vessels are larger, there are some simple perforation plates on the vessel members, there is some alternate intervascular pitting, the vessel elements are shorter, and the rays are homogeneous I.

The Platanaceae are also higher than the Stachyuraceae, Myrothamnaceae, and Buxaceae in all anatomical particulars.

This description of the woods of the Platanaceae does not differ very markedly from that of BRUSH (41).

EUCOMMIACEAE

The family Eucommiaceae contain the single species, *Eucommia ulmoides* Oliv. It is a tree of temperate China. The present study is based on 1 specimen with but 3 growth rings.

All the fibrous tracheary elements are tracheids. Vessel distribution is mostly solitary with a few pore multiples and clusters. There is a slight tendency toward ring-porosity in the wood. The pores are angular and thin-walled (fig. 42). The diameter of the pores is extremely small, rarely very small (range 20 to 34 μ; mean 27 μ). All the perforation plates on the vessel elements are simple. The end walls on the vessel members vary from 20° to 60°. Intervascular pitting is opposite with some alternate. Vessel element length is very short to medium sized, mostly medium sized (range 230 to 400 μ; mean 336 μ). There are spiral thickenings in the vessel members.

The rays are heterogeneous IIB, almost homogeneous I. The rays are uniseriate or biseriate. The former are 1 to 43 cells in depth and the latter from 4 to 32 cells.

The xylem parenchyma is diffuse and terminal (fig. 42). Vessel-parenchyma pitting is opposite and alternate.

The Eucommiaceae are higher than the Hamamelidaceae in that the former have vessel elements with simple perforation plates, there is some alternate intervascular pitting, the vessel elements are

shorter,[6] and the rays are heterogeneous IIB. The Eucommiaceae also have a higher type of vessel element than the Platanaceae.

Anatomically the Eucommiaceae are almost on the same level of specialization as are the Ulmaceae. They differ from the Ulmaceae in that they have tracheids and the parenchyma distribution is diffuse and terminal. Some of the Ulmaceae have vestigial scalariform perforation plates on the vessel elements also.

HYDRANGEACEAE

The family Hydrangeaceae consists of shrubs and trees. Their geographical distributional areas are chiefly temperate North America and eastern Asia. ENGLER and PRANTL (**62**) place 16 genera and some 150 species in this group. The present study is based on an investigation of 3 genera and 11 species. The genera studied are *Philadelphus* (shrubs) (3 spp.) (very young), *Deutzia* (shrubs) (4 spp.), and *Hydrangea* (shrubs or at times trees) (4 spp.).

Growth rings are present in 3 species. The other species were represented by specimens too young to show the rings.

There are tracheids and fiber-tracheids in 8 of the species. Two of these species have spiral thickenings in the tracheids and in the fiber-tracheids. One species has septate fiber-tracheids exclusively.

The pores are mostly solitary with a few multiples (fig. 43). Two genera are diffuse-porous and one is ring-porous. The vessels are angular (fig. 43). The walls of the vessels are thin in 10 species, and thin to thick in 1 species. Vessel diameters are extremely small to moderate sized, mostly very small (averages range 22 to 130 μ; mean of the family 55 μ). Tyloses are present in 1 species. Here they are thin-walled and few. The perforation plates on the vessel elements in all the species are exclusively scalariform. The plates have perforations with borders at the ends in 1 species and non-bordered in 10 species. All the species have many bars, except 1 species which has a few bars on the plates of vessel members. One species has wide perforations; all the others have narrow. The vessel element end walls vary from 5° to 65°. Intervascular and vessel-parenchyma

[6] Of course this study is based on young material; yet the vessel elements are so very much shorter than in the Hamamelidaceae that this statement is probably accurate.

pitting is scalariform and transitional in 1 species; scalariform, transitional, and opposite in 1; scalariform, transitional, opposite, and alternate in 3; and transitional, opposite, and alternate in 1. The vessel pits are small in 1 species. Vessel element length is medium sized to very long, mostly moderately long (averages range 560 to 1100 μ; mean of the family 946 μ). Spiral thickenings are present in the tips or "ligules" of vessel elements in 5 species. Two of the latter species have spiral thickenings in the tracheids and fiber-tracheids also. Two species have spiral thickenings throughout the vessel elements; that is, not restricted to the tips.

The rays are heterogeneous I in 2 genera and heterogeneous IIA in 1. In width, the rays vary from 1 to 6 cells. In depth, the uniseriates run from 1 to 26 cells and the multiseriates from 9 to several hundred. Some sheath cells are present in the rays of all 3 genera.

Xylem parenchyma distribution is diffuse in 1 species and diffuse and vasicentric in 4 others (fig. 43). Just a few cells are in contact with the vessels in the vasicentric type of parenchyma; that is, a continuous sheath is not formed about the vessels.

Anatomically the Hydrangeaceae are slightly higher than the Hamamelidaceae: most of the species have some fiber-tracheids, 1 form has septate fiber-tracheids, 1 species has thin to thick vessel walls, vessel diameter is larger, a few species have some alternate intervascular pitting, and the vessel elements are slightly shorter.[7]

Grossulariaceae

The Grossulariaceae are a family of shrubs found in the north temperate zone, on the mountains of Central America, and on the Andes of South America. The family is made up of 1 genus, *Ribes*, and about 140 species. The present study is based on an investigation of 4 species.

Growth rings are present in all. There are tracheids about the vessels, but the rest of the fibrous tracheary elements are septate fiber-tracheids.

Most of the vessels are solitary, some are in multiples, and a few are in clusters. All the species examined are ring-porous. The vessels

[7] Based on young material, however.

are angular and thin-walled (fig. 44). The vessel diameter is extremely small to very small, mostly extremely small (range 14 to 42 μ; mean 28 μ). The perforation plates on the vessel elements are exclusively scalariform in 3 species and scalariform and simple in 1 (fig. 61). In the latter group the vessels with simple perforation plates are very rare. All the species have non-bordered apertures. The bars on the plates are intermediate in number in 2 species and few in 2 species (fig. 61). Half the forms have narrow and the other half have wide perforations. The vessel element end walls vary from 5° to 40°. Intervascular and vessel-parenchyma pitting is scalariform (most), transitional, opposite, and alternate (rare). Vessel element length is medium sized (range 360 to 660 μ; mean 473 μ).

The rays are heterogeneous IIA. They are very wide, or so-called "compound," being from 1 to 22 cells in width. The uniseriate rays vary from 1 to 15 cells in depth and the multiseriates from 9 to 70 cells. Sheath cells are present in the rays of 2 species.

The xylem parenchyma is metatracheal, forming bands from 1 to 6 cells in width. These bands are quite rare, however.

The secondary xylem of the Grossulariaceae resembles very closely that of the Hydrangeaceae. The former is slightly more specialized in that all the species have septate fiber-tracheids, 1 species has some simple perforations on the vessel elements, the vessel member length is shorter, all the rays are IIA, and the parenchyma is metatracheal. In the Hydrangeaceae, however, the vessel diameter is greater and the vessel member end walls are not so oblique.

Escalloniaceae

The Escalloniaceae are a family of shrubs or trees occurring mainly in the southern hemisphere. There are 22 genera and about 100 species in this group. The following anatomical description is based on a study of 4 genera: *Brexia* (young), *Itea* (young), *Escallonia*, and *Carpodetus*.

Growth rings are present in 2 genera. Tracheids and fiber-tracheids are found in all 4 genera.

The vessels are mostly solitary with just a few multiples. All the species are diffuse-porous. The pores are angular and thin-walled

(fig. 45). Vessel diameters are extremely small and very small, mostly very small (average range 18 to 39 μ; mean of the family 30 μ). The perforation plates on the vessels are exclusively scalariform in 3 genera and simple with some vestigial scalariform in *Brexia*. In the 3 genera with scalariform perforation plates, the perforations are completely bordered in 1 genus and non-bordered in the other 2. All 3 genera have narrow apertures and many bars. The vessel member end walls vary from 5° to 55°. Intervascular pitting is scalariform, transitional, and opposite in 2 genera; transitional, opposite, and alternate in 1; and opposite and alternate in 1. The pits on the vessel elements are small in 3 genera. Vessel element length is medium sized to very long, mostly medium sized (averages range 445 to 1120 μ; mean of the family 687 μ). Spiral thickenings are found in the vessel members of 2 genera.

The rays are heterogeneous I in *Carpodetus*, heterogeneous IIA in *Escallonia*, and heterogeneous III in *Brexia* and *Itea* (both young). In width, the rays vary from 1 to 7 cells. *Carpodetus*, however, has very wide, or so-called "compound" rays. In the latter genus the rays vary from 1 to 20 cells in width. In depth, the uniseriate rays run from 1 to 53 cells and the multiseriates run from 5 to several hundred (in *Carpodetus*). There are some sheath cells in the rays of 2 genera.

Xylem parenchyma is diffuse and metatracheal in 1 genus and diffuse, vasicentric (fig. 45), and metatracheal in 1. In both genera the metatracheal parenchyma is in the form of irregular, uniseriate lines. The vasicentric parenchyma cells usually do not form continuous sheaths about the vessels. The xylem parenchyma is metatracheal (1 to 6 cells wide) in *Brexia*. No parenchyma was observed in *Itea* (young). Vessel-parenchyma pitting is scalariform, transitional, and opposite in 2 genera and transitional, opposite, and alternate in the other 2.

Crystalliferous chambered parenchyma cells are found in *Brexia*. Crystals are present in the ray cells of *Brexia* and *Carpodetus*.

Anatomically the Escalloniaceae are very close to the Grossulariaceae. The former perhaps may be considered to be a little higher, for 1 genus in the Escalloniaceae has vessel elements with simple and rarely vestigial scalariform perforation plates. On the other

hand, the Grossulariaceae are more specialized in regard to certain other characters.

The 3 families Hydrangeaceae, Grossulariaceae, and Escalloniaceae are very similar anatomically, but in general it may be said that the Hydrangeaceae are primitive, Grossulariaceae less so, and Escalloniaceae least primitive.

RECORD (**118**) has described the wood of *Escallonia tortuosa* H.B. and K. It varies but slightly from *E. rubra* Pers. which was examined by the writer.

BRUNELLIACEAE

The Brunelliaceae are a family of trees inhabiting the Andes from Mexico to Peru. ENGLER and PRANTL (**62**) list 1 genus with 17 species in the family. The present study is based on an investigation of several specimens of *Brunellia comocladifolia* Humb. and Bonpl.

No growth rings are found. The fibrous tracheary elements are fiber-tracheids.

There are slightly more solitary pores than pore multiples. The wood is diffuse-porous. The vessels are angular and thin-walled. Vessel diameters are very small and small, mostly small (range 36 to 82 μ; mean 62 μ). Tyloses are present and are thin-walled and few. The perforation plates on the vessel elements are scalariform and simple. The vessel elements with scalariform perforation plates have perforations bordered to the middle; the bars are intermediate in number and the apertures are wide. The end walls of the vessel members vary from 5° to 40°. Intervascular pitting is scalariform, transitional (fig. 59), and rarely opposite. The vessel elements are medium sized to very long, mostly moderately long (range 720 to 1480 μ; mean 1093 μ).

The rays are heterogeneous I; some specimens have only uniseriate rays and so the rays would be classed as heterogeneous III. The rays are 1 to 6 cells in width. The uniseriates run from 1 to 45 cells in depth and the multiseriates from 4 to 35 cells.

No xylem parenchyma was observed in any of the specimens. The vessel-parenchyma pitting (to the ray cells) is scalariform, transitional, and opposite.

The Brunelliaceae are higher than the Hamamelidaceae in the

following ways: presence of fiber-tracheids, larger vessel diameter, and presence of some vessel elements with simple perforation plates. The Brunelliaceae are also higher than the Hydrangeaceae on the basis of these 3 characters. However, the latter have less oblique vessel end walls, some alternate intervascular pitting, shorter vessel elements, and a higher type of ray in some species.

Cunoniaceae

The Cunoniaceae are mainly an Australasian family with 26 genera and about 250 species of trees and shrubs. Three genera and 12 species were examined in this study. The genera studied are *Ceratopetalum*, *Cunonia*, and *Weinmannia* (10 spp.).

Growth rings are present in 11 species. The fibrous tracheary elements in all the species are tracheids.

Most of the pores are solitary, with a few pore multiples and clusters in *Ceratopetalum*. In the other 2 genera the pores are mostly solitary with a few pore multiples. All the woods are diffuse-porous. Record (**122**) states that *Platylophus* is partially ring-porous. The vessels are angular and thin-walled in all species. The vessel diameters are extremely small to small, mostly small (averages range 38 to 69 μ; mean of the family 53 μ). A few thin-walled tyloses are present in 2 of the genera. The perforation plates of the vessel elements are exclusively scalariform in 2 genera and scalariform and simple in *Ceratopetalum*. The apertures in the scalariform perforation plates are bordered at the ends in 2 genera and non-bordered in *Weinmannia*. There are many bars in 2 genera and intermediate in number in 1. The apertures are wide in 2 genera and narrow in 1. Vessel end walls vary from 10° to 60°. The intervascular pitting is scalariform, transitional, and opposite in 3 genera. Vessel element length is medium sized to very long, mostly moderately long (averages range 761 to 1048 μ; mean of the family 925 μ).

The rays are heterogeneous IIA in 2 genera. However, they are almost heterogeneous I in *Weinmannia*. The rays are heterogeneous IIB in *Ceratopetalum*. In width, the rays vary from 1 to 4 cells. The uniseriate rays run from 1 to 26 cells in depth and the multiseriates from 4 to 38 cells.

Xylem parenchyma is metatracheal (1 to 3 cells in width) in

Ceratopetalum. In the other 2 genera it is diffuse and metatracheal (1 to 7 cells in width). Vessel-parenchyma pitting is scalariform and transitional in *Ceratopetalum* and scalariform, transitional, and opposite in the other 2 genera.

Chambered parenchyma cells with crystals are found in all the species.

The Cunoniaceae are on about the same level of anatomical development as are the Brunelliaceae. The former are lower in that all the species have tracheids, the vessel diameter is slightly smaller, and 2 genera have exclusively scalariform perforation plates. On the other hand, the Cunoniaceae have shorter vessel elements, the vessel member end walls are less oblique, and the rays are of a higher type.

Dichapetalaceae (or Chailletiaceae)

The Dichapetalaceae (or Chailletiaceae) are a family of small trees and shrubs occurring in the tropics, mainly tropical Africa. Engler and Prantl (**62**) list 4 genera and some 219 species in this family. The summary of the anatomy given here is based on a study of *Tapura cubensis* Griseb.

Growth rings are present. The fibrous tracheary elements are fiber-tracheids.

Most of the vessels are in multiples; there are some solitary pores and a few clusters. The wood is diffuse-porous. The vessels are angular and thick-walled. The vessel diameters are usually extremely small with some very small (range 30 to 52 μ; average 41 μ). The perforation plates are scalariform and simple. There are about an equal number of plates in each class. The perforations on the scalariform perforation plates are non-bordered and wide. There is an intermediate number of bars on the plates. Vessel end walls vary from 10° to 50°. The intervascular and vessel-parenchyma pitting is alternate. The pits on the vessel elements are very small. Vessel element length is medium sized to very long, mostly moderately long (range 620 to 1150 μ; average 895 μ).

The rays are heterogeneous I and from 1 to 2 cells wide. The uniseriates vary from 1 to 45 cells in depth and the multiseriates from 9 to 62 cells.

Xylem parenchyma distribution is metatracheal (uniseriate lines). The ray cells bear many crystals.

ENGLER and DIELS (**60**) and BESSEY (**33**) place the Dichapetalaceae in the Geraniales. WETTSTEIN (**158**) assigns the family to the Tricoccae near the Euphorbiaceae. Since the woods of these other groups have not been examined by the writer, and since the anatomical description of the Dichapetalaceae is based on such an inadequate sample of the family, no attempt is made to dispose of the family at this time. However, the Dichapetalaceae are on about the same level of anatomical specialization as are the Cunoniaceae and Brunelliaceae—perhaps just a little higher in a few characters.

The description of the wood given here is in accord with that published by RECORD (**117**).

ROSACEAE

The Rosaceae are an extensive family of trees, shrubs, and herbs divided into some 90 genera and into about 2000 species. They have a cosmopolitan distribution, but are found mainly in the north temperate zone. The present investigation is based on a study of 36 genera and 68 species. The genera studied include *Physocarpus* (shrubs) (young), *Neillia* (shrubs) (young), *Stephanandra* (shrubs) (young), *Spiraea* (shrubs) (young), *Vauquelinia* (shrubs and small trees), *Exochorda* (shrubs) (young), *Cotoneaster* (shrubs) (2 spp.) (young), *Pirus* (trees and shrubs) (6 spp.), *Eriobotrya* (trees), *Photinia* (shrubs and small trees), *Pourthiaea* (shrubs), *Amelanchier* (shrubs and small trees) (2 spp.), *Mespilus* (shrubs or small trees) (young), *Rubus* (shrubs) (4 spp.) (some young), *Potentilla* (shrubs and herbs) (2 spp.) (young), *Fallugia* (shrubs), *Cowania* (shrubs), *Purshia* (shrubs), *Chamaebatia* (shrubs) (young), *Rosa* (shrubs) (6 spp.) (young), *Pygeum* (trees and shrubs) (2 spp.), *Prunus* (trees and shrubs) (10 spp.), *Prinsepia* (shrubs), *Chrysobalanus* (shrubs and small trees) (2 spp.), *Licania*, *Hirtella* (shrubs or small trees), *Parinarium* (trees) (4 spp.), *Acioa* (trees and shrubs), *Lyonothamnus* (trees), *Aronia* (shrubs) (young), *Chaenomeles* (shrubs) (young), *Cercocarpus* (trees and shrubs), *Crataegus* (small trees and shrubs) (2 spp.), *Heteromeles* (shrubs), *Micromeles* (trees) (2 spp.), and *Opulaster* (shrubs). The anatomical characters of the young ma-

terial were not tabulated, unless this material showed striking differences in anatomy from the other specimens.

Growth rings are found in 20 species. Two species, *Prunus caroliniana* Ait. and *P. domestica* L., have vertical intercellular canals of the traumatic type.

The fibrous tracheary elements in 3 genera are all tracheids. In 23 genera there are tracheids and fiber-tracheids—mostly tracheids in the greater number of genera. In 4 genera the tracheids and fiber-tracheids have spiral thickenings.

Vessels are mostly solitary, with a few pore multiples in all but 1 genus. In the latter there are mostly solitary pores with a few multiples and rarely clusters. Three genera and 1 species of *Rosa* have ring-porous woods. *Opulaster* and 4 species of *Prunus* have semiring-porous woods. The rest of the genera have diffuse-porous woods. The vessels are angular (fig. 46) in 12 species and angular to round (fig. 47) in 16 species. Vessel walls are thin in 27 and thick in 1 species. Vessel diameter is extremely small to rather large (fig. 47), mostly very small and small (averages range 21 to 218 μ; mean of the family 70 μ). A few thin-walled tyloses are present in 3 species. The perforation plates on the vessel elements are simple, with very few scalariform plates in 17 species. In 14 of this class of 17 species, some of the plates are reticulate (fig. 60), or are modifications of the reticulate arrangement (fig. 58). BLISS (**34**), MACDUFFIE (**106**), and THOMPSON (**142**) have described and photographed these reticulate and modifications of reticulate plates in the Rosaceae. In 3 of the 17 species no reticulate plates are seen. In 2 species the plates are simple and vestigial scalariform and in 9 species they are all simple. In the forms with scalariform perforation plates, the perforations are completely bordered in 2 species and non-bordered in 13; the bars are intermediate in number in 1 and few in 14; and the apertures are narrow in 1 and wide in 14. Vessel element end walls vary from 10° to 90°. Three species have some vessel element end walls at 90°. The intervascular pitting is transitional (rare), opposite (some), and alternate (most) in 2 species. It is alternate in 7 and in all the other forms it is opposite and alternate. Vessel element length is extremely short to moderately long, mostly medium sized (averages range 193 to 757 μ; mean of the family 487 μ). Spiral thickenings are present in the vessel elements

of 21 species. Three of these 21 species also have spiral thickenings in the tracheids and fiber-tracheids.

The rays are heterogeneous I in 3 species, heterogeneous IIA in 7, heterogeneous IIB in 9, heterogeneous III in 2, and homogeneous I in 5. The rays in the 5 forms with homogeneous I rays are nearly heterogeneous IIB. The rays vary from 1 to 8 cells in width. One form, *Rubus australis* Forst., has very wide, or so-called "compound" rays (from 1 to 50 cells in width). In depth, the uniseriate rays are from 1 to 42 cells and the multiseriates from 3 to 84 cells, except in 2 forms in which the rays run to many hundreds of cells. There are a few sheath cells in 1 species.

Xylem parenchyma distribution is diffuse in 2 species; diffuse and vasicentric[8] in 10; diffuse, terminal (1 cell wide), and vasicentric[8] in 2 (fig. 46); diffuse, metatracheal (in irregular uniseriate lines), and vasicentric in 5; metatracheal (1 to 4 cells wide) and vasicentric (mostly complete sheaths) in 5; and diffuse and confluent in 1 (fig. 47). Vessel-parenchyma pitting is opposite and alternate in 14 species and alternate in 9. Unilateral compound pitting is found in 4 species. Pith flecks are seen in 5 species.

Crystals are present in the ray cells of some species. Crystalliferous chambered parenchyma cells are found in 6 species.

Only a few (4) herbaceous stems were examined. However, EAMES (**57, 59**), SINNOTT and BAILEY (**133**), and JEFFREY and TORREY (**89, 90**) have published descriptions of the anatomy of other herbs in the Rosaceae. As a result of this work, it may be said that many of the herbs of this family are of the "continuous" type while some have discrete bundles.

The Rosaceae are more specialized anatomically than are the Cunoniaceae. This is apparent for in the Rosaceae there are fiber-tracheids, angular to round vessels, larger pores, some species with simple perforations on the vessel elements, some forms with vessel end walls at 90°, shorter vessel members, some alternate intervascular pitting, and higher types of rays.

CALYCANTHACEAE

The Calycanthaceae are a small family of shrubs occurring in temperate eastern Asia, in North America, and in tropical Aus-

[8] Not in the form of sheaths, just 2 or 3 cells next to the vessels.

tralia. The family consists of 1 genus and 5 species. A young piece of *Calycanthus mohrii* Small was available for anatomical study.

Growth rings are present. The fibrous tracheary elements are fiber-tracheids.

The vessels are mostly in the form of multiples with some solitary pores. The wood is ring-porous. Vessels are angular and thin-walled. Vessel diameter is extremely small to very small, mostly extremely small (range 16 to 36 μ; average 27 μ). All the perforation plates on the vessel members are simple. Vessel end walls vary from 10° to 65°. The intervascular and vessel-parenchyma pitting is alternate. Vessel element length is moderately short to medium sized, mostly medium sized (range 330 to 500 μ; average 414 μ). Spiral thickenings are present in the vessel elements.

The rays are 1 to 2 cells wide and are heterogeneous I. The uniseriate rays vary from 1 to 35 cells in depth and the multiseriates from 5 to 30 cells.

Xylem parenchyma distribution is vasicentric, not in the form of a continuous sheath about the vessels, but just 2 or 3 parenchyma cells in contact with the vessels.

The Calycanthaceae are rather highly specialized anatomically. The perforation plates are simple, the intervascular pitting is alternate, the fibrous tracheary elements are fiber-tracheids, and the vessel elements are fairly short.[9] Admittedly this anatomical description is based on too poor a sample of the family to allow any convincing generalization to be made on its relationship. It can be said, however, that the derivation of the Calycanthaceae from the more primitive members of the Rosaceae would not be inconsistent with the anatomical facts. Yet it must be remembered that certain groups in the Magnoliales (the Himantandraceae and Lactoridaceae) have simple perforation plates on the vessel elements, and alternate intervascular pitting also.

Aristolochiaceae

The Aristolochiaceae are a family of herbs or woody vines distributed widely in both temperate and tropical parts of the world. Engler and Prantl (**62**) list 7 genera and some 381 species in this

[9] Based on young material, however.

family. The anatomical notes are based on a study of 4 species of *Aristolochia*. Two of these species showed no secondary tissues.

Growth rings are present. The fibrous tracheary elements are tracheids and fiber-tracheids.

The vessels are solitary, in multiples and in clusters. The wood is ring-porous in 2 species (fig. 48). The vessels are angular to round with thin to thick walls. Vessel diameters are small to large, mostly rather large. The perforation plates are simple. Some of the vessel element end walls are 90°. Intervascular pitting is alternate. Vessel element length is extremely short to moderately short, mostly very short.

The rays are homogeneous and up to 40 cells wide. These very wide rays dissect the stem into discrete bundles (fig. 48). The rays may extend from one internode to another.

The xylem parenchyma is vasicentric and metatracheal.

SOLEREDER (**136**) has described the anomalous structure of the axis in *Aristolochia triangularis* Cham. In this species the original vascular ring is split up and a fan-shaped stele is produced.

It is apparent that the Aristolochiaceae are very highly specialized herbs and vines. The vessel elements are very large and quite short, and the wood is ring-porous. The rays are exceedingly wide and very deep and so the stele is divided up into a number of discrete bundles.

Discussion

PHYLOGENY

Interpreting the anatomical data given for each family in the light of the lines of structural specialization discussed in the introduction, together with a consideration of the facts from floral and general morphology, the writer has come to certain conclusions concerning the phylogenetic relationships of the various orders and families studied in this investigation. These conclusions are summarized in figures 62 and 63.

Figure 62 is an attempt to depict the levels or planes of anatomical specialization, as well as the phylogeny of the several groups. The chart is divided into four zones, beginning at the bottom of the page with a zone containing the families with exclu-

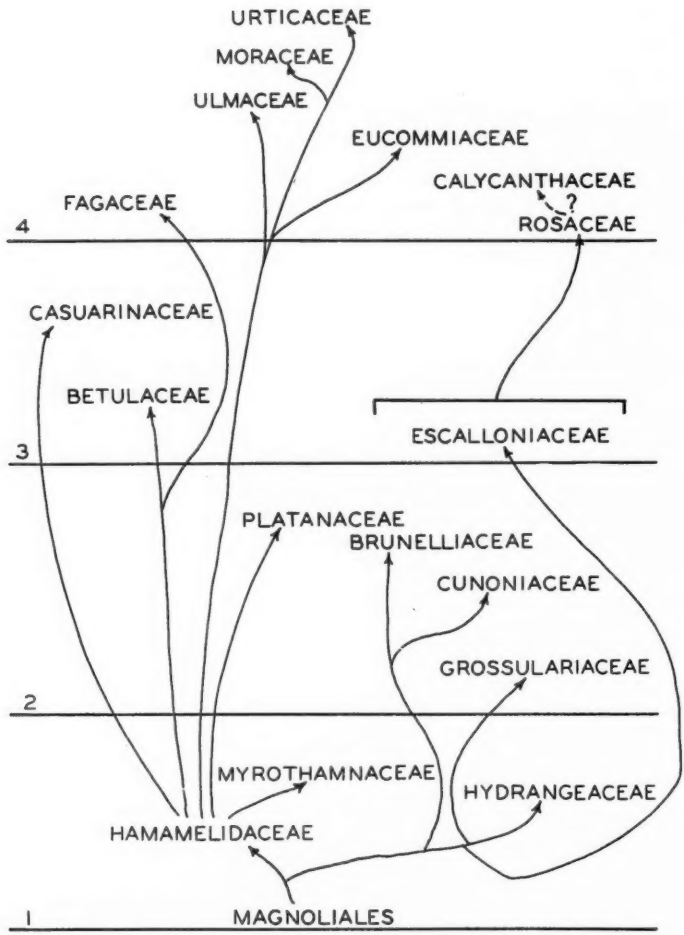


FIG. 62.—Proposed phylogenetic relationships of families investigated in this study: (1) exclusively scalariform perforation plates; (2) scalariform and simple perforation plates; (3) simple and vestigial scalariform perforation plates; and (4) simple perforation plates.

sively scalariform perforation plates on the vessel elements, next the zone or region with the families characterized by scalariform and simple perforation plates, still higher the zone containing the families with simple and vestigial scalariform perforation plates,

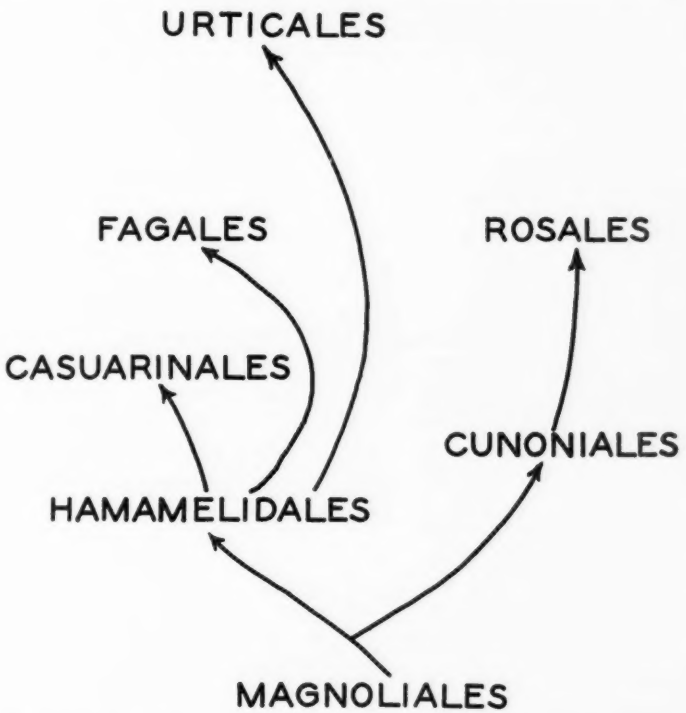


FIG. 63.—Proposed phylogenetic relationships of orders investigated in this study

and finally the region with the families which have simple perforation plates on the vessel elements. The relative positions of the families within the various regions are governed by the degree of anatomical specialization of the families. Thus two families may have exclusively scalariform perforation plates, yet one may have shorter vessel elements, a more primitive type of intervascular pitting, and a lower type of ray. This family, then, would appear

lower in the "exclusively scalariform" region than the other family. Some of the families, of course, exhibit considerable variation among the genera and species as to the type of perforation plate. In such cases the families are placed on the level of the highest members of the group. For example, in the Cunoniaceae certain forms have vessel elements with exclusively scalariform perforation plates, whereas certain other species have vessel members with simple and scalariform perforation plates; therefore the Cunoniaceae are placed in the region containing the families with scalariform and simple perforation plates.

The phylogenetic relationships expressed in these charts and in the text are not, of course, considered to be final. They merely represent the writer's conclusions based on the evidence at his disposal at the present time. Further evidence from the fields of cytology, paleobotany, anatomy, and taxonomy may alter considerably the conclusions reached in the present investigation.

Since in most cases the anatomical reasons for placing one family near or above another have already been stated in the summarizing paragraph at the conclusion of the anatomical description of each family, only a few supplementary comments on the anatomical evidence, together with a brief discussion of the facts from general external morphology, especially floral morphology, need to be added. Finally, the evidence from floral anatomy, paleobotany, nodal anatomy, and cytology will be discussed.

It has already been indicated that certain groups in the Magnoliales have a very primitive array of anatomical characters. The Magnoliales exhibit a considerable range of anatomical specialization, yet the more primitive groups (anatomically) may be characterized as follows: Some of the members have no vessels. Many of the forms have exclusively scalariform perforation plates. The perforations are completely bordered, or bordered to the middle, or bordered at the ends. Many species have many bars on the perforation plates; often the perforations are narrow. The vessel elements are characterized by great length and small diameter. The vessels are usually angular and thin-walled. The vessel end walls are very oblique. Intervascular pitting is scalariform in many. The woods are diffuse-porous. Most of the vessels are solitary. The fibrous

tracheary elements in many species are tracheids exclusively. The fibrous tracheary elements are very long. Many of the forms have heterogeneous I rays. Some of the species have diffuse parenchyma. Almost all forms are arboreal in habit. On the basis of anatomy, therefore, there is much to recommend the Magnoliales as a very primitive order among the dicotyledons. Likewise, from the standpoint of floral morphology, many phylogenists have considered the Magnoliales as the most primitive group, or among the most primitive groups of the flowering plants (**31, 32, 33, 77, 79, 83, 3, 153**). Those who regard the Magnoliales as a primitive group stress the following floral and external vegetative characters of that order: The flowers are hypogynous and hermaphroditic. The floral parts are often spirally arranged. There are many stamens which are usually free. There is much endosperm and the embryo is minute. The leaves are simple and usually alternate. ENGLER and PRANTL (**61, 62**) and WETTSTEIN (**158**), of course, dissent from the view that the Magnoliales are the most primitive order of the dicotyledons. WETTSTEIN thinks of the Magnoliales as being derived from some of the apetalous groups. ENGLER, in his later writings, feels that the Amentiferae and the Ranales (including the Magnoliales) are derived independently from a hypothetical group which he calls the Protangiospermae. Unlike WETTSTEIN, ENGLER does not say that the Magnoliales have evolved from the Amentiferae, but he does indicate that he believes that the Amentiferae possess more primitive characteristics than the Ranales. From the anatomical evidence, however, it would appear that the Magnoliales are primitive and the Amentiferae (at least the Casuarinales, Fagales, Juglandales, and Urticales) are more highly specialized.

Figure 62 pictures the Hamamelidaceae as a derivative of the Magnoliales. In the summary at the close of the anatomical description of that family it was indicated that the Hamamelidaceae are very close to the Magnoliales anatomically. Yet the Hamamelidaceae seemed a little higher. HALLIER (**78, 79**) derives the Hamamelidaceae from the Magnoliaceae. LOTSY (**105**) follows HALLIER in this respect. WETTSTEIN (**158**) also sees a relationship between the Hamamelidales and the Polycarpicae (containing the Magnoliales). His system begins with the Amentiferae, however, and so he

places the Hamamelidales before the Polycarpicae. ENGLER and PRANTL and BESSEY picture the Rosales (containing the Hamamelidales) as coming from the Ranales, but they do not regard the Hamamelidaceae as the primitive family in the Rosales. It is obvious, then, that with the exception of WETTSTEIN, all those who do not derive the Hamamelidaceae directly from the Magnoliales, or Ranales (containing the Magnoliales), at least derive the Rosales (with the Hamamelidaceae) from the Ranales. In other words, from the standpoint of the flower there is nothing against the idea that the Hamamelidaceae came from the Magnoliales. The flowers of the Hamamelidaceae show the following advances over those of the Magnoliales: Often the flowers are unisexual, even dioecious. Sometimes the flowers are naked. The calyx at times is adnate to the ovary. The flower may be hypogynous to epigynous. The stamens are reduced in number. There are usually 2 carpels which are free at the apex. The ovules vary in number from many to 1.

Figure 62 indicates that the Myrothamnaceae had their origin in the Hamamelidaceae. Almost all phylogenists consider the Myrothamnaceae as being very close to the Hamamelidaceae. That the former are more advanced, or at least as highly advanced, as the more specialized members of the Hamamelidaceae is evidenced by the fact that in the former the flowers are dioecious, the spikes are catkin-like, the calyx and corolla are absent, and the stamens are reduced to 4–8.

The Platanaceae are clearly more advanced than the Hamamelidaceae anatomically. In all the systems the Platanaceae are placed in the immediate neighborhood of the Hamamelidaceae. Florally the former seem more advanced, or at least as highly advanced as the highest members of the Hamamelidaceae, in that the flowers in the Platanaceae are monoecious and in heads. The perianth is much reduced or may be lacking. The stamens are reduced to 3–8. There is usually 1, or rarely 2, ovules in the 1-celled ovary. The flowers are wind-pollinated.

It is obvious that in the families of the Hamamelidales (Hamamelidaceae, Myrothamnaceae, and Platanaceae) there are many tendencies which are very suggestive of the Amentiferae. Among these, mention might be made of the tendency toward unisexual

and naked flowers, the tendency to form heads and even catkin-like spikes, and the tendency to produce 2 carpels with 1 or 2 ovules. HUTCHINSON (**84**) has pointed out that the characteristic type of leaf common to many members of the Amentiferae is also found in the Hamamelidaceae.

No final deposition of the Stachyuraceae and the Buxaceae is attempted here for the reasons already indicated. However, no anatomical evidence was found which would militate against the placement of these two families in the Hamamelidales as derivatives of the Hamamelidaceae. Further study of the other suggested relatives of these two families may, of course, alter the situation.

The Eucommiaceae are much more specialized anatomically than are the Hamamelidaceae. In fact they are almost on the level with the Ulmaceae. In most of the systems of phylogeny, the Eucommiaceae are placed next to the Hamamelidaceae. WETTSTEIN, in the earlier editions of his Handbuch der systematischen Botanik, also classified the Eucommiaceae in the Hamamelidales. In his fourth edition (**158**), however, he puts the Eucommiaceae in the Urticales. H. HARMS, in the second edition of Die natürlichen Pflanzenfamilien (**62**), sees some characters of this family which remind him of the Hamamelidaceae and some which strongly suggest the Urticales. He concludes that "es läszt sich nicht leugnen, dass die Gattung (*Eucommia*) ebenso gut oder vielleicht noch besser in der Reihe der Urticales untergebracht werden könnte." As for external morphology, the flowers are dioecious and without a perianth. The carpels are 2 in number, 1 usually aborting. The ovules are 2 in number, pendulous and anatropous. The fruit is samaroid. There are latex tubes in certain parts of the plant. The Eucommiaceae are here regarded as being an offshoot from the main line of evolution in the Urticales, near the Ulmaceae. The Eucommiaceae are a very interesting and significant family, for they share many characters with the Hamamelidaceae on the one hand, and with the Urticales on the other.

Figure 62 depicts the Casuarinaceae as originating from the Hamamelidaceae. This is very possible as far as anatomical evidence is concerned, for the Casuarinaceae are higher than the Hamamelidaceae in every anatomical character. HUTCHINSON (**83**) derives

the Casuarinaceae from the Hamamelidales. In 1924 (**82**) and again in 1926 (**84**) he made clear that it was the Hamamelidaceae from which the Amentiferae originated. BESSEY (**33**) states that the Hamamelidaceae gave rise to the Casuarinaceae. PARKIN (**112**) likewise thinks that the Amentiferae may have been derived from the Hamamelidaceae. Indeed many of the floral characters of the Casuarinaceae are shared by some of the Hamamelidaceae. Among these characters, mention may be made of the monoecious and dioecious conditions, of the female flowers borne in heads, of the lack of perianth, and of the presence of but 2 carpels and 2 ovules. In addition, in such undoubted relatives of the Hamamelidaceae as the Myrothamnaceae, one finds male flowers in spikes and in the Platanaceae there is wind-pollination. On the other hand, ENGLER and PRANTL and WETTSTEIN consider the Casuarinaceae as very primitive dicotyledons. Essentially their arguments are four: presence of many megaspores, chalazogamy, wind-pollination, and absence of a perianth (or at most a bractlike perianth) in the Casuarinaceae. However, many megaspores have been discovered in the Ranales, in the Rosales, in the Fagales, and in other orders. Significantly, several megaspores have also been found in *Hamamelis*[10] (**131**). Likewise chalazogamy has been found in many other groups. Indeed intermediate conditions between chalazogamy and true porogamy have been discovered, so that chalazogamy has lost much of its significance, for one may argue as have COULTER and CHAMBERLAIN (**51**) that chalazogamy is derived from true porogamy through such intermediate conditions as is found in *Ulmus*, *Cucurbita*, and *Alchemilla*. Further, ROBERTSON (**128**) has presented evidence to show that anemophilous flowers are derived from entomophilous ones. Lastly, the Casuarinaceae are considered primitive because they have no perianth, or at most a bractlike perianth. But it has already been indicated that certain members of the Hamamelidaceae have no perianth. Also, the Myrothamnaceae and Eucommiaceae have flowers with no perianth. The Platanaceae have

[10] SHOEMAKER also found that fertilization took place 5 to 7 months after pollination in *Hamamelis*. This delay between fertilization and pollination, because of the similarity to the situation in the gymnosperms, is often cited as proof that some of the Amentiferae are primitive.

flowers with no perianth, or a much reduced one. In these families the perianth-less condition is usually considered a derived state. This appears to be true from the evidence obtained from studies in floral anatomy (**36**). If the lack of perianth in these families can be explained by reduction, there seems to be little to prevent the interpretation of the absence of a perianth in the Casuarinaceae in the same way. Future investigations of the floral anatomy of the Casuarinaceae no doubt will settle this matter. Certainly the highly specialized secondary anatomy of the stem would indicate a reduced rather than a primitive family.

Figure 62 indicates that the Fagales had their origin in the Hamamelidaceae. On the basis of secondary wood anatomy this seems quite likely, for the Betulaceae (anatomically the primitive family in the order) are more highly specialized than the Hamamelidaceae in all anatomical characters. HUTCHINSON derives the Fagales from the Hamamelidales. ARBER and PARKIN (**3**) are inclined to believe that the Fagales came from the Hamamelidaceae. Floristically the Fagales could have evolved from the Hamamelidaceae, for many of the characters of the Fagales are also seen in the Hamamelidaceae. Some of these characters are: monoecious flowers, rarely dioecious, or bisexual; calyx reduced or absent; stamens 2 to many; inferior ovary; 2 to 6 united carpels; and 1 to 2 ovules. Other characters of the Fagales are possessed by certain undoubted relatives of the Hamamelidaceae; that is catkin-like spikes. On the other hand, ENGLER and PRANTL and WETTSTEIN consider the Fagales as primitive largely on the basis of the simple perianth (sometimes absent), chalazogamy, and wind-pollination. This perianth may be reduced, however, as the floral anatomy indicates (**2**). Chalazogamy also seems to have lost much of its phylogenetic significance, as has been already suggested. Further, there seems to be good reason to believe that wind-pollination may be a derived condition. Finally, the anatomy of the secondary xylem of the stem is highly specialized. This evidence added to that from floral anatomy and that from floral morphology would seem to indicate that the Fagales are reduced and not simple plants. And the available facts at hand seem to point to their derivation from the Hamamelidaceae.

The Juglandales are not included in figures 62 and 63 for there

is good reason to believe that they, on the basis of their pinnate leaves, resinous condition, and other characters, might well have been derived from one of the groups in the Sapindales. Certainly the secondary xylem would indicate that the Juglandaceae are rather a high group and not a simple one as WETTSTEIN considers them. But since the xylem of the Sapindales was not studied in this investigation, no final deposition of the Juglandaceae is attempted at this time. However, HUTCHINSON, BESSEY, and HALLIER derive them from groups in the Sapindales.

Figures 62 and 63 picture the Urticales arising from the Hamamelidaceae, or at least some hamamelidaceous ancestor. From the anatomical viewpoint this origin for the Urticales is the most likely one of all the possible ancestors investigated, for the vestigial scalariform perforation plates in some members of the primitive family Ulmaceae indicate that the Urticales are to be derived from some group with scalariform perforation plates. ENGLER and PRANTL consider the Urticales as more or less primitive forms derived from the Protangiospermae. WETTSTEIN pictures them as arising close to the Fagales and Juglandales. He (**157**) does, however, see affinity between the Hamamelidaceae and the Urticales. This affinity is further borne out by the fact that he places the family Eucommiaceae, considered by nearly everyone else as close relatives of the Hamamelidaceae, in the Urticales. HUTCHINSON derives the Urticales from the Hamamelidales. GRIGGS (**75**) finds that the Platanaceae, considered by all to be very close to the Hamamelidaceae, resemble the Urticales in many ways. BESSEY derives the Urticales from the Tiliaceae and so considers them as members of the Malvales. HALLIER (**77**) considers them as derivatives of the Sterculiaceae through the Euphorbiaceae, the Rhamnaceae, or the Tiliaceae; subsequently he (**79**) changed his mind and derived them from the Terebinthaceae.

The writer has examined slides of the secondary xylem of 14 genera and 23 species of the Tiliaceae and 14 genera and 32 species of the Sterculiaceae. On the basis of this study, it is concluded that these two families did not give rise to the Urticales. The two families are very highly specialized anatomically. In the Sterculiaceae all the vessel elements have simple perforation plates. Twelve species are

found with storied structure. The pores are rather large in many species. The family also contains herbs and shrubs in addition to woody climbers and trees. In the Tiliaceae, all the vessel elements have simple perforation plates. Fifteen species have storied structure. Most of the species have libriform wood fibers. There are shrubs and herbs in this family also. The fact that some of the species in the Ulmaceae have vessel elements with vestigial scalariform perforation plates would seem to imply that this family was derived from some group with scalariform perforation plates.

The xylem of the Terebinthaceae has not been examined in this investigation, therefore it cannot be said with certainty that the Urticales are not derived from this family. LOTSY (**105**), however, in a critical analysis of HALLIER'S system (which he favors for the most part), points out that HALLIER'S derivation (**78**) of the Amentiferae (including the Urticales) from the Terebinthaceae is based on rather feeble evidence. Indeed HALLIER himself (**79**) made this very significant statement, after summarizing the evidence for deriving the Amentiferae and the Urticales from the Terebinthaceae: "Malgré ces circonstances on ne doit pas encore pour le moment nier la possibilité d'une autre descendance des plantes à chatons. *Mais il n'y a pas d'autres ancêtres possible que les Hamamélidacées, ou encore, . . . les Rosacées . . . . La question pourra probablement être tranchée par l'étude comparée du bois*" (italics mine).

On the whole, HUTCHINSON'S idea of tracing back the Urticales to the Hamamelidales seems to fit best with the anatomical data. From the standpoint of external morphology there is much to recommend this view. The following characters of the Ulmaceae, the primitive family in the Urticales, are shared by them and by some members of the Hamamelidaceae: The leaves are alternate and simple. The flowers are bisexual or unisexual. The calyx is 4- to 8-lobed and the lobes are imbricate. The ovary is superior with 1 to 2 united carpels containing 1 to 2 cells. There are 2 styles. There is 1 ovule, pendulous from near the top of the cavity of the ovary. The stamens are few in number, and the anthers which open lengthwise are 2-celled.

However, according to HUTCHINSON some of the Urticales are to be derived from the Aristolochiales. Presumably these are the

herbaceous members which are derived from the herbaceous side of his phylogenetic tree. Anatomically there is not the slightest evidence for this diphylesis of the Urticales. The Urticales are very homogeneous anatomically. The herbaceous forms are exactly like their woody relatives; or more correctly perhaps, like the first growth layers of their woody relatives, in all anatomical details. In the Urticaceae the herbs even have the odd heterogeneous I rays of their woody neighbors. *Urtica dioica* L. has even the unlignified areas which occur in many of the woody members of the family. Apparently it would be stretching the concept of convergent evolution rather far in attempting to argue that the herbaceous forms coming from the Aristolochiales would be identical in the flower and even in the minutest details of anatomical structure with those woody forms derived from the Hamamelidales. The herbaceous species studied were clearly derivatives of the woody forms. Furthermore, there was no similarity between the anatomy of the Aristolochiales and that of the Urticales. Indeed, the Aristolochiaceae[11] (fig. 48) are much more specialized anatomically than are the herbs of the Urticales. The pores are larger, the vessel elements are shorter, and some forms are even ring-porous (fig. 48). The rays are very wide (fig. 48) in the Aristolochiaceae and so the herbs and vines are divided up into discrete bundles, whereas in the herbaceous forms of the Urticales the rays are narrow and the herbs are of the "continuous" stele type.

Figure 62 pictures the Ulmaceae as primitive, the Moraceae less so, and the Urticaceae as least primitive. Anatomically this is certainly true. With the exception of WETTSTEIN, the phylogenists likewise depict the same sequence of families. The evidence from external morphology also shows this sequence to be true. In the Ulmaceae the leaves are alternate. The flowers are perfect, polygamous, monoecious, or rarely dioecious. The calyx is subcampanulate and the lobes are from 4 to 9 in number. The stamens are equal to and opposite the calyx lobes, or there may be more stamens than the number of calyx lobes. The ovary is superior and made up of

[11] The Aristolochiales, according to HUTCHINSON, include the Aristolochiaceae, Cytinaceae, Hydnoraceae, and Nepenthaceae. But the last 3 families are obviously very specialized derivatives of the Aristolochiaceae and so their anatomy need not be considered.

2 carpels with 2 styles. There are 1 to 2 cells, usually 1, in the ovary. There is 1 anatropous ovule which is pendulous from the apex of the ovary. The seeds are without endosperm. The embryo is straight. In the Moraceae the leaves are alternate, rarely opposite. The flowers are monoecious or dioecious. The calyx is from 4- to 5-lobed—sometimes less, or lacking altogether. The stamens are equal to and opposite the calyx lobes. The ovary is superior usually and has 2 carpels, one of the latter not developing as a rule. The styles are 2 or 1. There is usually 1 ovule, sometimes 2, to each ovary. The ovules are anatropous and pendulous from the apex or rarely basal. The seeds are with or without endosperm. The embryo is often curved. In the Urticaceae the leaves are alternate or opposite. The flowers are monoecious, dioecious, or rarely polygamous. The calyx is 4-lobed, at times 5-lobed. The stamens are equal to and opposite the calyx lobes. The ovary is superior and is made up of 1 carpel with 1 cell. There is 1 style and 1 ovule. The ovule is "orthotropus" and erect at the base.[12] The seeds have endosperm mostly. The embryo is straight. It is apparent that in the 3 families there is a general trend from bisexual to monoecious to dioecious flowers, from many sepals to few, and from 2 carpels to 1. There is also a change in the position of the ovule.

ENGLER and PRANTL (**61**) seem to consider that the sub-family Moroideae are nearer to the Ulmaceae than are any of the other sub-families of the Moraceae. The tribe Fatoueae of this sub-family, especially, is mentioned by them as being very similar to the Ulmaceae. It has already been stated that anatomically the Moroideae are on the whole the most primitive sub-family in the Moraceae. The tribe Fatoueae also, on the available anatomical evidence, seems to have a good claim to being considered the most primitive tribe in the family, for the Fatoueae have neither septate-fibers nor latex tubes in the rays.

As previously stated, the Rhoipteleaceae obviously do not belong in the Urticales. HANDEL-MAZZETTI (**80**) points out that this family differs from the Ulmaceae in that the Rhoipteleaceae have compound leaves and the ovule is not pendulous.

[12] The true nature of this ovule will be elucidated in connection with the discussion of floral anatomy.

Figures 62 and 63 show the Cunoniales (including the Hydrangeaceae, Escalloniaceae, Cunoniaceae, Brunelliaceae, and Grossulariaceae) arising from the Magnoliales. The Cunoniales in turn are shown giving rise to the Rosales (including the Rosaceae, and perhaps the Calycanthaceae). Almost all the phylogenists derive the Rosales (including the families of the Cunoniales[13]) directly from the Ranales. Likewise nearly all these writers picture the families here included under the order Cunoniales as primitive, and they depict the Rosaceae as derived. Since this is the accepted view among taxonomists, no detailed discussion of the floristics of the various families is necessary. Anatomically it can be said that the Cunoniales are primitive and the Rosales are derived. No attempt has been made to determine from which of the families in the Cunoniales the Rosales were derived. Trends toward the Rosales can be seen in the Cunoniaceae and in the Hydrangeaceae, Grossulariaceae, and Escalloniaceae. Indeed the last three groups could very well be placed in one family.

Evidence from paleobotany

A survey of the paleobotanical literature (**23, 24, 25, 26, 27, 28, 29, 30, 46, 97, 98, 103, 114, 161, 104, 92, 93, 140**) pertaining to the families under consideration sheds but little light on the problem of phylogeny. The meager information which can be gleaned from this source is not convincing, for a good many of the families under investigation in the present study seem to have been already present in the rocks of the Cretaceous period. Further confusion results from the fact that many of the fossils in the Upper Cretaceous are merely leaves. As a result, a considerable proportion of these early records are subject to doubt and others are certainly erroneous. Accordingly only a brief summary will be made of the fossil evidence.

Arbitrarily, the families may be divided into three groups on the basis of their geological histories. The first group includes the families whose fossil records extend back beyond, or are abundantly represented in, the Upper Cretaceous. These families are the Magnoliaceae, Hamamelidaceae, Platanaceae, Fagaceae, Betulaceae, Juglandaceae, Moraceae, and Anacardiaceae. Many species

[13] Most investigators—Engler and Prantl, Wettstein, Bessey, and Hallier—include the families of the Cunoniales in the Rosales.

of the Magnoliaceae, based on leaf material, are found in the Upper Cretaceous. There is some indication that this family was present in the Lower and the Middle Cretaceous periods. SAHNI (**129**) has described *Homoxylon rajmahalense*, a wood which resembles the homoxylous Magnoliales (especially *Trochodendron*), in that there are no vessels, from the Jurassic of India. If this record is definitely established, it will greatly strengthen the position of the Magnoliales as a primitive angiospermous order (**76**). Both leaves and fructifications of the Hamamelidaceae have been removed from beds of the Upper Cretaceous. The leaves of Hamamelites are reported from the Mid-Cretaceous. LIGNIER (**104**) has described *Hamamelidoxylon* from the same horizon. Many leaves and a few fruits of *Platanus* (Platanaceae) are found in the Mid-Cretaceous. *Plataninium* (wood of the Platanaceae) has been described from the Upper Cretaceous. Leaves of many of the Fagaceae are present in the Upper Cretaceous. *Betula* leaves have been discovered in the same period but the other genera of the Betulaceae seem to make their first appearance in the Eocene. The leaves of *Juglans* (Juglandaceae) are found in the Upper Cretaceous, and there is a suggestion that they may be present in the Middle Cretaceous. Wood from this family under the name of *Juglandinium* is listed from the Upper Cretaceous. Fossil leaves of the Moraceae seem to be fairly common in the Upper Cretaceous. Without question many of these leaves have been incorrectly assigned to the genus *Ficus*, yet undoubted leaves and fruits of *Ficus*, and similarly leaves, fruits, and flowers of *Artocarpus*, are certainly found in this period. Some 10 to 12 species of *Rhus* (Anacardiaceae) have been described from the Upper Cretaceous.

The second group of families consists of those which are found in the Upper Cretaceous but are not abundant there. The Ulmaceae, Sterculiaceae, Sapindaceae, and Aristolochiaceae seem to belong in this category. The Ulmaceae are found in the Upper Cretaceous but are not so well represented as the families listed under the first group. The Sterculiaceae and the Sapindaceae have a few forms attributed to them from this geological horizon. Leaves and fruits of the Aristolochiaceae have been uncovered in deposits of the Upper Cretaceous.

The third group is made up of families whose earliest record is in

the Tertiary. The Casuarinaceae are mainly Tertiary, although one report places them as far back as the Mid-Cretaceous. The oldest known members of the Tiliaceae are from the Eocene. The Hydrangeaceae are first found in the Eocene. The Cunoniaceae appear in the Miocene and the Grossulariaceae occur in the Pleistocene for the first time. The Rutaceae have been found in the Lower Tertiary. Records of the Rosaceae are largely from the Tertiary. A few seem to extend back into the Upper Cretaceous. The Buxaceae have leaves and fruits in the Pliocene.

From the paleontological evidence at hand it cannot be said with any degree of certainty that the Amentiferae or the Magnoliales are primitive. Representatives of both groups are seemingly present in the Upper Cretaceous. Yet BERRY (**27**) and THOMAS (**140**) have both called attention to the fact that the Magnoliaceae are very abundant in the Upper Cretaceous. SAHNI'S discovery of the Jurassic *Homoxylon* seems to indicate also that the Magnoliales may with good reason be considered a primitive group from the standpoint of paleobotany. However, it seems clear that if the Amentiferae were derived from some primitive angiosperm complex, this evolution must have occurred early. There is little in the available fossil evidence which would seem to oppose the notion that the Urticales, the Fagales, and the Casuarinales are derived from the Hamamelidaceae. Indeed the latter family seems to have a fair claim of being Mid-Cretaceous. Certainly it can be concluded that the paleobotanical facts are in accord with the derivation of the Urticales from the Hamamelidaceae, rather than from the Anacardiaceae, or from the Sterculiaceae, or from the Tiliaceae.

It is odd that the Ulmaceae should appear to come later than the Moraceae. This situation is probably due to the rather questionable nature of some of the early records of *Ficus* leaves. Actually *Planera* (Ulmaceae) is found in the Upper Cretaceous and there are some reports of *Ulmus* from the same period.

The Cunoniales and Rosales seem to be definitely later than the Magnoliales.

Emphasis must again be placed on the fact that the fossil record is very incomplete, and therefore any conclusions based on this evidence are of doubtful validity. Future discovery and investiga-

tion of angiospermous fruits and wood from the Lower Cretaceous and Jurassic will no doubt remove much of the uncertainty.

Evidence from nodal anatomy

The research of Sinnott (**132**) has shown that there are three fundamental types of nodal organization in the angiosperms. The primitive, or trilacunar condition, is one in which there is a foliar supply of three bundles, each causing a gap in the stem cylinder. Next there is the condition where a single gap is left by the foliar supply, whether the latter is single or multiple. This type of nodal structure is designated unilacunar, and has been derived from the trilacunar state, either through the fusion of the two lateral traces with the central one, accompanied by the disappearance of the separating segments of the stele, or by the abortion of the two lateral strands. Finally there is the type of nodal structure known as the multilacunar condition. Here the foliar supply consists of many bundles, each causing a gap in the cylinder. The multilacunar type is thought to be derived from the primitive trilacunar type by amplification.

Sinnott (**132**) lists the following nodal conditions for the groups under consideration in the present study:

Magnoliales	3, 1, and many	Moraceae	3 and 5
Hamamelidaceae	3	Urticaceae	3
Stachyuraceae	3	Eucommiaceae	1
Buxaceae	1	Hydrangeaceae	3 and (5 or 7)
Platanaceae	7	Cunoniaceae	3
Casuarinaceae	1	Brunelliaceae	3 and 5
Betulaceae	3	Rosaceae	3 and (1 or 5)
Fagaceae	3	Calycanthaceae	3
Ulmaceae	3		

Other families which have figured in the discussion of the phylogeny of the groups investigated have nodal conditions as follows:

Anacardiaceae	3	Aristolochiaceae	3
Juglandaceae	3 and (5)	Sapindales	3 and (5)
Tiliaceae	3	Rutaceae	3 and (1)
Sterculiaceae	3		

It can be seen that the proposed phylogeny of the various groups as pictured in figures 62 and 63 is in accord with the facts of nodal

anatomy. As SINNOTT remarks, the Ranales (including the Magnoliales) seem to have had the trilacunar condition primitively and the other types in that order were derived from this trilacunar condition. The Hamamelidaceae are trilacunar, and thus the various groups with the trilacunar, unilacunar, or multilacunar nodes could be considered to be derived from this family.

It must be admitted, however, that several of the other phylogenetic schemes receive as much support from nodal anatomy as does the one suggested in this paper. For example, from the evidence SINNOTT gives, the Urticales might be derived from the Sterculiaceae, or the Tiliaceae, or the Anacardiaceae. Taken alone, therefore, the evidence from nodal anatomy means little, but when it is placed beside the testimony of secondary xylem anatomy, of floral morphology, of paleobotany, and of other fields of research, it is not without import perhaps. Two facts stand out from the work on nodal anatomy, which even considered alone have some significance. First, the nodal organization of the Ranales is more variable than are most orders, and so SINNOTT has suggested that this variability or plasticity is an indication of the primitiveness of the Ranales. Second, the Casuarinaceae are unilacunar, and are therefore eliminated as a possible primitive group.

EVIDENCE FROM FLORAL ANATOMY

BECHTEL (19) has made an intensive study of the floral anatomy of the Urticales. As a result he found that in the flowers of the Ulmaceae there is evidence that an inner whorl of stamens and an inner whorl of perianth parts, supposedly petals, have been suppressed in the course of the evolutionary history of the family. (Also EAMES, 58.) He discovered conditions suggesting that formerly the carpels contained many ovules; also that there was slight evidence that the flowers of *Ulmus* might once have had more than 2 carpels. Anatomically the floral parts are spirally arranged; that is, the vascular traces to the floral parts are spirally arranged. He cites evidence which indicates that some of the perianth parts and some of the stamens have been suppressed in the 2 whorls which remain. The flowers are slightly zygomorphic, and BECHTEL suggests that this type of symmetry and the evidence of the multiovulate condi-

tion mean that the family had an entomophilous ancestor. The stamens are fused to the gamophyllous perianth. The number of floral parts varies from flower to flower in a given species. The male flowers of *Celtis* bear an abortive pistil, indicating derivation from a bisexual ancestor.

BECHTEL thinks that the Ulmaceae cannot be considered a very primitive group, therefore, for they show reduction in whorls, in members of a whorl, in the number of carpels, and in the number of ovules in a carpel. They are characterized by zygomorphy, syncarpy, the fusion of like parts (the sepals are fused to form the gamophyllous perianth), and the fusion of unlike parts (the stamens are fused to the perianth). On the other hand, he warns against placing the Ulmaceae too high, for the flowers have the spiral arrangement (anatomically) and the floral parts are variable in number.

In the present paper the Urticales are derived from the Hamamelidaceae. Therefore it will serve a useful purpose to test out the Hamamelidaceae as a possible ancestor of the Urticales with the anatomical facts reported by BECHTEL. Some members of the Hamamelidaceae possess an inner row of stamens and a corolla. Other forms have many ovules and are zygomorphic. Some of the species of the Hamamelidaceae have more than 5 sepals, petals, and stamens. Characteristically a calyx tube is present in the flowers. The flowers of the Hamamelidaceae are insect-pollinated as a rule. The flowers of the family do not have more than 3 carpels, but perhaps this loss of carpels took place in geological history at a time when the Hamamelidaceae and the Urticales had not become differentiated as such. Future floral anatomical studies of the Hamamelidaceae will reveal whether this family also has indications of previous polycarpous condition. There seems to be some doubt that these bundles in the Urticales actually prove the earlier existence of polycarpy (**19,** pp. 396, 400). All in all, the Hamamelidaceae seem to qualify as the ancestral group of the Ulmaceae.

In the Moraceae BECHTEL found that there were traces in the staminate flowers which indicated the earlier presence of a pistil. In other words, these unisexual flowers seem to be derived from bisexual ones and are not simple. In the forms with but 1 style he found vascular bundles of another.

In the Urticaceae there is only 1 carpel, but BECHTEL found vascular traces which imply the former presence of another carpel. He also found evidence of a pistil in the male flowers. Further, he settled the problem of the position and type of ovule in this family and in some members of the Moraceae. In the Ulmaceae and in some of the Moraceae, the ovule is anatropous and pendulous from the apex of the ovary. But in the other forms of the Urticales, the ovule is basal and "orthotropous," according to the usual interpretation. It has always been a problem to explain this basal, "orthotropous" ovule in a family which is supposedly higher than the Ulmaceae where the ovule is anatropous and pendulous. BECHTEL found that the vascular trace supplying the basal ovule goes up to the apex of the ovary and then bends down to the basal ovule. He therefore interprets this ascending and descending course of the trace to mean that the primitively pendulous and anatropous ovule of the Ulmaceae and some Moraceae has slid down to a basal position, and in this sliding down process the anatropous ovule has become erect to form a falsely "orthotropous" ovule.

BECHTEL concludes that from his studies the Ulmaceae are primitive, the Moraceae less so, and the Urticaceae least primitive. We have seen that this same sequence is indicated by the anatomy of the secondary xylem of the three families.

BOOTHROYD (**36**) has investigated the morphology and the anatomy of the flower and of the inflorescence of the Platanaceae. She finds that the perianth is much reduced. The calyx may be reduced to a mere cup, or may be lacking altogether in certain male flowers. The petals may be vestigial organs or absent. The male flowers have rudimentary carpels at times and the staminodia in the female flowers are clearly sterile stamens. The inflorescence is much reduced. All these facts point to the derivation of the flowers of the Platanaceae from bisexual, insect-pollinated flowers with well developed calyx and corolla. The Platanaceae are interesting for they resemble the Amentiferae in many ways, and they indicate how the flowers of the latter group may have lost their perianths and become unisexual in connection with modifications for wind-pollination.

There is nothing in BOOTHROYD'S work which would militate against the derivation of the Platanaceae from the Hamamelidaceae

except the number of carpels. Since there are several free carpels in the Platanaceae and 2 (or rarely 3) fused carpels in the Hamamelidaceae, the former are not to be regarded as derivatives of forms like the modern Hamamelidaceae, but as derivatives of some common ancestor which possessed several free carpels.

ABBE (**2**) has investigated the floral and inflorescence anatomy and morphology of the Betulaceae. He found that the inflorescence is much reduced by loss of bracts and of florets. Fusion of bracts has also occurred. In the florets the perianth or perigon may be present, vestigial, or absent. If the perianth is lacking, traces indicating the former presence of tepals are found. The stamens may be reduced to 1.

ABBE'S study shows conclusively that the Betulaceae are not simple plants but very much reduced ones. His work accords with the derivation of the Betulaceae from the Hamamelidaceae. He suggests that the ancestral condition for the gynoecium in the Betulaceae might have been tricarpellary. Some of the members of the Hamamelidaceae have 3 carpels, so this condition is also satisfied by the Hamamelidaceae.

BERRIDGE (**22**) has studied the morphology and anatomy of the Fagaceae, and has found that in certain forms the female flowers bear stamens and the male flowers contain a rudimentary ovary. Also she found vascular conditions which suggest that the flowers of *Castanopsis* once possessed a whorl of petals. Some evidence was seen which signified that the tricarpellary ovary had been reduced from a pentamerous gynoecium.

She concludes that the Fagaceae were probably derived from some primitive angiospermic stock with flowers characterized by bisexuality, syncarpy, multilocular ovaries (pentamerous possibly), and epigyny. These ancestral flowers had both calyx and corolla. On the basis of these facts she selects the Rosales (specifically the epigynous Rosaceae, or their near descendents) as the groups with closest affinity to the ancestors of the Fagaceae. Nothing in her work offers serious opposition to the derivation of the Fagaceae from the Hamamelidaceae, however, except the number of carpels. So it may be that the ancestral stock from which both the Hamamelidaceae and the Fagaceae evolved had more than 3 carpels.

MANNING (**107**) has made a brief report on his research on the Juglandaceae. He finds evidence of reduction in the inflorescence and in the flowers, and so concludes that the pro-juglandaceous stock had terminal panicles of perfect flowers.

In this connection it might be added that rudimentary ovaries have been found in the male flowers of some members of the Juglandaceae (**83**). Also, BENSON and WELSFORD (**20**) have published evidence that the ovule in this family is not basal and orthotropous, but is parietal and anatropous. As a result of these investigations, it seems clear that the Juglandaceae are much reduced plants.

EVIDENCE FROM CYTOLOGY

The haploid chromosome numbers[14] of the groups investigated in the present study are as follows:

Magnoliales	14, 19, 38, 48
Hamamelidaceae	12, 15, 24, 36
Platanaceae	8, 10–11, 21
Casuarinaceae	12
Betulaceae	8, 11, 14, 21, 28, 32, 35, 42
Fagaceae	11, 12, 13, 24
Ulmaceae	14
Moraceae	8, 9, 10, 12, 13, 14, 15, 16, 28
Urticaceae	7, 12, 13, 14, 16, 24
Hydrangeaceae	10, 11, 13, 14, 16, 18, 65
Grossulariaceae	8, 10
Escalloniaceae	11, 32
Rosaceae	4, 6, 7, 8, 9, 10, 14, 16, 17, 21, 26, 28, 32
Calycanthaceae	10, 11, 12
Juglandaceae	16, 17, 32
Aristolochiaceae	7, 14

The haploid chromosome numbers in certain groups which have figured in the discussion on phylogeny are as follows:

Rutaceae	8, 9, 13, 18, 35
Tiliaceae	8, 9, 41
Sterculiaceae	8
Sapindales	5, 7, 11, 12, 13, 14, 15, 20, 26, 36, 40

[14] These chromosome figures have been taken from GAISER, **67, 68, 69**; TISCHLER, **144, 145**; WANSCHER, **149**.

WANSCHER (**149**) has shown that chromosome numbers in plants may be divided into five kinds of evolutionary series: "(1) The uniform series: for example 7-7-7-7. (2) The multiple series: 7-14-21-28, etc. (3) The descending series: 8-7-6-5. (4) The ascending series: 8-9, etc. (5) To the fifth class we may perhaps refer the uncertain and irregular secondary polyploid series; for example the series 7-14-17 in Pomaceae."

In the absence of other cytological evidence, it is difficult to determine whether one is dealing with an ascending or with a descending series in a given set of chromosome numbers. Also, the mere fact that two groups have the same chromosome number need not imply that the two are closely related. However, if evidence from floral morphology, stem anatomy, and other fields of research indicate a possible affinity between groups of plants, frequently the evidence from chromosome counts offers useful corroborative testimony. In this paper a certain phylogenetic scheme has been proposed; therefore it is interesting to see how the cytological facts fit in with the rest of the data.

It seems clear that the basic numbers, 12 and 15, of the Hamamelidaceae could have been derived from the 14 of the Magnoliales. Similarly the basic numbers of the Casuarinales, Fagales, and Urticales could be derived from the Hamamelidaceae by the methods WANSCHER has outlined.

Future cytological research on the morphology and behavior of chromosomes will no doubt establish the basic numbers in the various taxonomic units. Then it will be possible to make much wider use of these basic numbers in discussions of phylogeny. Even now a beginning has been made in this type of cytological investigation, but since the families have not as yet been studied extensively, it seems best to await further results.

General conclusions

As a result of the study of these families, it seems evident that in general the evolution of the flower has been correlated with the evolutionary development of anatomical structures. Thus it is that after the rearrangement of certain questionable groups, the classi-

fication of the taxonomist and the phylogenetic scheme of the anatomist show surprising agreement.

At this time it must be admitted that the use of the anatomical method in phylogenetic studies has certain definite limitations. BAILEY (12) has pointed out that similar combinations of anatomical characters may occur in families which are widely separated as to systematic position. He makes special mention of the remarkable resemblance between the xylem of *Maclura aurantiaca* Nutt. of the Moraceae and the wood of *Robinia pseudoacacia* L. of the Leguminosae. Therefore, if the anatomist were to build up a phylogenetic system on the basis of anatomy alone, some rather strange results would ensue. Because of this convergent evolution, and also because of parallel evolution in stem structures, the anatomist must proceed with caution.

The anatomist can, however, make certain definite contributions to problems of phylogeny if he uses the various systems built upon the basis of floral morphology as a background for his work. For example, in the present study we begin with the various placements of the Urticales of the several phylogenists before us. Then the theories of each phylogenist were tested out with the anatomical facts.

The suggestions of the anatomist can be of even greater value if he harmonizes his conclusions based on anatomy and floral morphology with the facts of paleobotany, floral anatomy, nodal anatomy, and cytology.

With the judicious employment of the methods outlined here, there is every indication that these methods will be of great value in the establishment of a natural classification of the angiosperms. Certain questionable groups may be assigned to their proper phylogenetic place. For example, the anatomy of the Rhoipteleaceae reveals that they do not belong in the Urticales. The compound leaves of the plants of this family lend support to this conclusion. A second type of service which the anatomist may render the phylogenist is assistance in the establishment of proper sequence of groups. HUTCHINSON, for example, shows the Magnoliales giving rise to the Cunoniales, the latter group giving rise to the Rosales, and finally

the Rosales giving rise to the Hamamelidales. Now the anatomical facts show that the Hamamelidaceae are very close to the Magnoliales, and so it is better to regard the Hamamelidales as being derived directly from the Magnoliales. Other lines of evidence seem to support this conclusion.

While not directly connected with the subject of the applicability of the anatomical method to problems of phylogeny, a number of other matters may be brought together at this time.

This survey of the anatomy of 22 families seems to lend support to the idea that non-septate fibrous tracheary elements (fiber-tracheids or libriform wood fibers) are primitive and the septate type of fiber (fiber-tracheid or libriform wood fiber) is derived. It was found that the former are usually present in structurally primitive woods, whereas the latter are present most often in rather highly specialized woods.

Since the solitary pore arrangement of vessels is found in primitive woods (such as those of the Hamamelidaceae), this solitary arrangement appears to be primitive, and the various aggregate arrangements, such as pore multiples, pore chains, and pore clusters, are derived types of vessel pattern.

There seemed to be some indication that the advent of ring-porosity, or of some factor or factors causing ring-porosity, gives an impetus to anatomical specialization. For example, in the Urticales the ring-porous woods of the Ulmaceae are more highly specialized than the diffuse-porous woods of the same family or of the Moraceae. Yet the Ulmaceae seem lower than the Moraceae from the viewpoints of floral morphology and floral anatomy. Anatomically too, the Ulmaceae on the whole seem less specialized than the Moraceae. If the ring-porous woods of the Moraceae are compared with the ring-porous woods of the Ulmaceae, the former are higher. Conversely, if the diffuse-porous woods of the Moraceae are compared with the diffuse-porous woods of the Ulmaceae, the former are higher. It will be recalled that BAILEY and TUPPER (**18**) found that the decrease in length of tracheary elements and the specialization of xylary structures was closely correlated with the advent and subsequent evolution of the vessel elements in the dicotyledons.

The anatomical investigation of the Moraceae and Urticaceae revealed that in comparative anatomical studies the secondary xylem of herbs and of shrubs must be compared with the secondary xylem of trees taken from a region close to the pith. If this is not possible, a correction must be made in the interpretation of ray type, vessel element length, vessel diameter, pore outline, and other structures in the essentially "youthful" herbs and shrubs.

Summary

1. There appears to be a correlation between the presence of sclerotic wood parenchyma cells in the xylem and the presence of sclerotic ray cells, sclerotic tyloses, and thick-walled fibers and vessel elements.

2. The advent of ring-porosity, or of some factor or factors causing ring-porosity, seems to give an impetus to anatomical specialization.

3. Non-septate fibers are more primitive than are the septate type of fibers.

4. The solitary pore arrangement of vessels is more primitive than the various aggregate arrangements, such as pore multiples, pore chains, and pore clusters.

5. In comparative anatomical studies, the secondary xylem of herbs and shrubs must be compared with the secondary xylem of trees taken from a region close to the pith.

6. The Hamamelidaceae are derivatives of the Magnoliales.

7. The Casuarinales, Fagales, and Urticales cannot be considered as primitive groups among the dicotyledons. These three groups are considered to be derivatives of the Hamamelidaceae.

8. The Cunoniales (including the Hydrangeaceae, Grossulariaceae, Escalloniaceae, Cunoniaceae, and Brunelliaceae) are derivatives of the Magnoliales. The Rosales have been derived from some group in the Cunoniales.

9. In the Urticales the Ulmaceae are most primitive, the Moraceae less so, and the Urticaceae are least primitive. The Eucommiaceae are also placed in the Urticales.

10. In the Moraceae anatomical specialization seems to have pro-

ceeded from the sub-family Moroideae to the Artocarpoideae to the Conocephaloideae to the Cannaboideae. The tribe Fatoueae of the sub-family Moroideae appears to be the most primitive one in the family.

11. There is no evidence for, and much against, the derivation of the herbaceous Urticales from the Aristolochiaceae.

12. The Rhoipteleaceae do not belong with the Urticales.

13. In general, HUTCHINSON'S system is more in accord with the phylogenetic scheme proposed in this paper than are the other systems. His division of the dicotyledons into a herbaceous line and an arboreal line is not borne out by the facts revealed in the present investigation.

14. In general, the evolution of floral structures seems to be correlated with the evolutionary development of anatomical structures. After the re-arrangement of certain questionable groups, therefore, the classification of the taxonomist and the phylogenetic scheme proposed by the anatomist show striking agreement.

15. There is every indication that the study of anatomy will be of great value in the establishment of a natural classification of the angiosperms.

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DEVELOPMENT AND STRUCTURE OF THE WATERMELON SEEDLING

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 493

GAYLE N. HUFFORD

(WITH FORTY-NINE FIGURES)

Introduction

BARBER (1) studied the fruits and seeds of the watermelon, *Citrullus vulgaris* Shrad. In addition to histological investigation, she determined that the cells of the embryo contain oil and protein granules as reserve food. Later, LANGELD (7) compared the oil content of seeds of various crop plants and found that those of watermelon were highest in this respect, ranking above those of pumpkin and soy beans. Using root tips as material, WHITAKER (12) determined the chromosome number of several of the cultivated Cucurbitaceae. He reported eleven as the haploid number for *Citrullus*. CROCKER and associates (2) considered the peg to be a local overgrowth of the cortical cells, and demonstrated that such overgrowth is stimulated mainly by the arching of the hypocotyl. HOLROYD (4) made comparative histological studies of several of the Cucurbitaceae, particularly of the mature stems. He described *Citrullus* as "excelling all other members of the family in length of axis, luxuriant growth of side branches, and in size of hypocotyl and immediately connected regions above and below." He also considered the sieve tubes to be the largest of any cucurbits studied, those of the external phloem averaging twice the size of those of the internal. SLEETH (11) has shown that the formation of tyloses in *Citrullus* is incited by *Fusarium niveum*. Plants one to four months old showed an abundance of tyloses, the abundance being correlated with the quantity and nearness of the fungus. The evidence indicates that the formation of tyloses precedes or coincides with the spread of the fungus through the xylem. HOLROYD (4) and RUTLEDGE (10) have reviewed the extensive literature dealing with the nature of the bicollateral bundles in the Cucurbitaceae.

It is the purpose of this investigation to show some of the developmental and structural features of the early stages of the seedling.

Material and methods

Plants were grown in the field and in the greenhouse, and very young seedlings were germinated in moist cotton. Since the peg normally causes the hypocotyl to emerge at a sharp angle with respect to the primary root, it is difficult to obtain strictly transverse serial sections in the transition region. This difficulty was overcome by germinating the seeds in an erect position, micropylar end downward, and with the seed coats partially removed after the general method used by CROCKER (2). Most seeds so prepared were germinated in moist cotton, since it was easier to secure and maintain an erect position by wrapping the seeds in moist cotton pads and inserting the pads in shell vials. Some seeds so prepared were also grown satisfactorily in the greenhouse.

The best results for all young tissues were obtained when they were fixed in a Navashin solution made up as follows: (A) 10 cc. glacial acetic acid, 1.5 gm. chromic acid, and 90 cc. distilled water; (B) 40 cc. formalin and 60 cc. distilled water. Equal amounts of the two solutions were mixed just before using. Older tissues were fixed in formalin-alcohol.

Chloroform was used to clear all material to be sectioned. Cedar oil was used to clear seedlings for macroscopic study of the vascular system. Seedlings for this purpose were killed in formalin-alcohol and left in the solution until the chlorophyll had been dissolved from the tissues. They were then split along one side of the hypocotyl to the pith cavity, laid open, and clamped between two microscope slides. So held they were dehydrated in alcohol and cleared with cedar oil. Serial sections for the study of histogens of the root were cut at 6μ; all others at 12μ. Flemming's triple stain was used.

Investigation

GERMINATION AND SEEDLING

The mechanics of germination are practically identical with those described by CROCKER (2) for other Cucurbitaceae. After the emergence of the primary root, the peg forms rapidly, so that by

the time the root is 2–3 cm. long it has fastened itself firmly against the lower half of the seed coats, while the simultaneous arching of the hypocotyl splits the halves apart. The opposing pull of the hypocotyledonary arch against the peg pulls the cotyledons from the integuments. These are left beneath the soil, often clamped over the peg. Rapid growth of the lower hypocotyl forces the arch upward through the soil and above ground. Continued growth results in the cotyledons being rapidly pulled from the soil. The arch straightens out and the cotyledons spread apart. Although yellowish or white at the time of emergence, the cotyledons and the aerial portions of the hypocotyl develop chlorophyll. It is only when seeds are planted on the micropylar end that the seed coats are carried above the ground by the cotyledons.

The time required for germination varies greatly. The seeds are very sensitive to temperature and water conditions. In warm greenhouse conditions the radicle usually emerges within 36–48 hours, and the cotyledons will be out of the ground and spread apart within four days. Growth of the hypocotyl is rapid. Within eight to ten days from time of planting it will have attained a length of 9–11 cm., which is about its maximum in an optimum environment. During this time there is a correspondingly rapid growth of the primary root and its branches, and of the cotyledons. The latter are ovate and have long and short diameters of about 11 and 7 mm. at the time they appear above the soil. Within a few days they reach maximum size, the average long and short diameters being 4 and 2 cm. respectively. These data relate to greenhouse-grown plants; under field conditions the periods of growth will be lengthened in proportion to the departure from optimum atmospheric conditions.

Full expansion of the cotyledons marks a definite stage in the growth of the plant, since two weeks to a month may elapse before further appreciable lengthening of the axis occurs. This stage is arbitrarily referred to as the seedling, of which this study covers only the earlier stages; that is, before marked secondary thickening has taken place.

At the time the cotyledons first spread apart the epicotyl is hardly visible. The point of the first regular leaf is deeply set in the angle between the cotyledons. The latter are densely spinulose

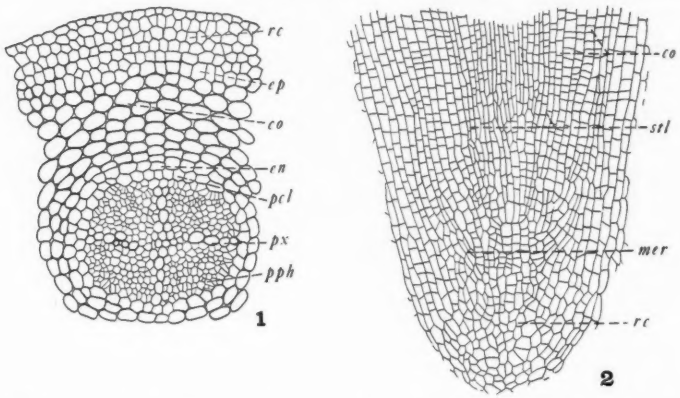
above and glabrous beneath, and each has seven main veins, more prominently displayed on the lower surface. The hypocotyl is somewhat flattened in the plane of the cotyledons and tapers slightly as it approaches them. It ranges from 3 to 4 mm. in diameter just above the peg to 1–1.5 mm. just below the cotyledons. Its surface is beset with spinulose hairs which increase in number and size from the ground upward. The primary root grows rapidly and apparently continues to do so throughout the life of the plant. It tapers abruptly just below the peg, and from it arise many laterals, definitely four ranked.

Primary root and transition

The primary root is tetrarch exarch. Its general organization is evident early in the ontogeny of the axis. Figure 1 shows a transverse section less than 1 mm. from the tip of a very young seedling in which the primary root had attained a length of approximately 5 mm. The protoxylem has begun to differentiate and shows as a cross within the pericycle. The primary phloem is somewhat more advanced in its development. In fact it is characteristic that the phloem precedes the xylem in development, and also exceeds it in amount formed. The pericycle is uniformly one cell thick opposite the phloem, except that there is usually an extra layer two to four cells wide adjacent to the protoxylem. The cells of the pericycle are large and angular in cross section. The endodermis can be determined by position only, since its cells approximate the size and shape of the cortical cells. No Casparian strips are visible. The cortex is from eight to ten cells thick. The cells are very large, oval, and somewhat compressed periclinally. The intercellular spaces are many. The epidermis is composed of one layer of cells, which are more or less rectangular in cross section. Around the epidermis at this level there are four or five layers of the root cap which have not been abraded. The cells resemble those of the epidermis, and compactly surround them. With the exception of the outer cap cells, all are highly meristematic, as is evidenced by their thin walls, prominent nuclei, and dense cytoplasm. The protoxylem is becoming definitely differentiated.

Figure 2 shows a median longitudinal section through the root

tip. As indicated, there are no definite histogens. At the apex is a common meristematic region from which all tissues are derived. This meristem lies in a horizontal plane at the base of the central cylinder, but outward from the cylinder it is turned up slightly, so that it has the shape of a shallow bowl. All stelar and cortical tissues are derived from cells cut off to the inside of this bowl-shaped promeristem. Cells cut off to the outside form the calyptra. Cells are cut off centripetally from the upturned edges of the meristem.



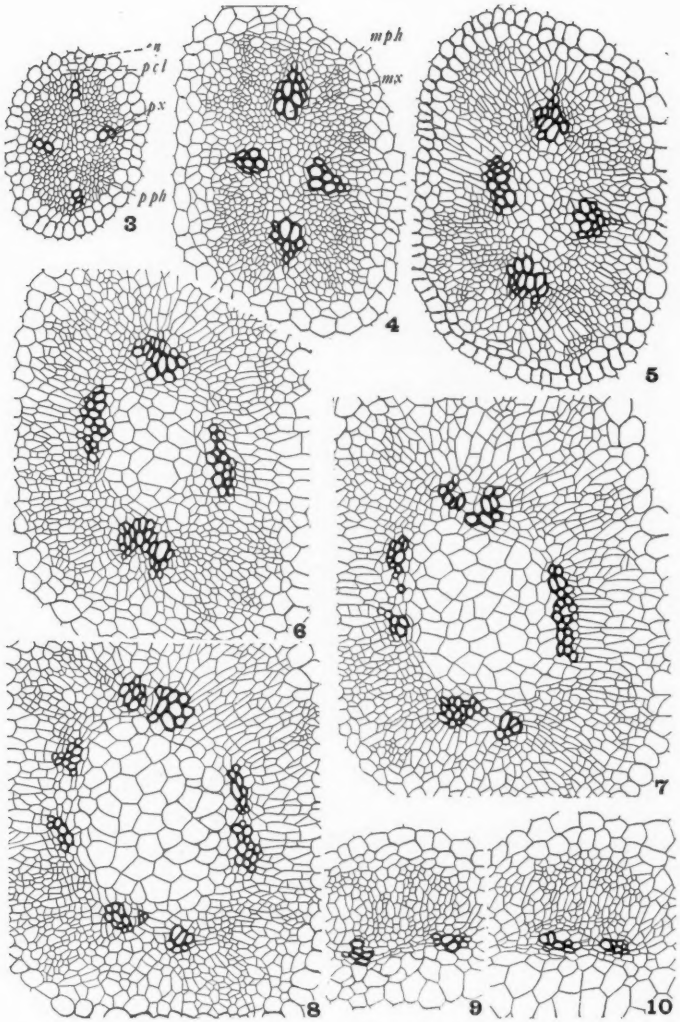
FIGS. 1, 2.—Sections from meristematic region of primary root: Fig. 1, cross section 2 mm. back from tip showing early differentiation of tissues (*rc*, root cap; *ep*, epidermis; *co*, cortex; *en*, endodermis; *pcl*, pericycle; *px*, protoxylem; *pph*, protophloem). Fig. 2, median longitudinal section through tip showing nature of unorganized meristem (*stl*, stele; *mer*, meristem). ×27.

Because of this the cap does not break away from the root, but adheres closely to it for considerable distance. Continued, although irregular, breaking away of the cap cells eventually leaves the innermost layer of these cells, which were cut off externally from the meristem, as the epidermis. As a consequence of its irregular method of derivation, the epidermis usually presents a rather uneven surface instead of the smooth and even surface of an epidermis derived from a definite dermatogen. This meristematic region of *Citrullus* is similar although not identical with the type of meristem described by JANCZEWSKI (5) as being common among the Cucurbitaceae and the Papilionaceae. He considered the upturned outer limits of the

meristem the transverse generating layer from which the central cylinder and the cortex are derived to the inside, and the calyptra to the outside. He also described the manner in which the epidermis is developed. The description given thus far relative to figures 1 and 2 applies to the tip region regardless of the age or the length of the root. In slightly older roots, 15–20 mm. long, it is possible to follow the development of the primary root by examining identical structures at successively higher levels.

Figure 3 shows a section about 8 mm. back from the tip. The protoxylem is well differentiated, being composed of annular and spiral elements. The metaxylem has begun to differentiate centripetally from the protoxylem. The primary phloem shows little greater differentiation. The elements of each of the four groups tend to be divided into two smaller groups toward the adjoining protoxylem points, with prominent parenchyma cells between each group. There is no marked change in the pericycle. The endodermis is well marked by Casparian strips, which are more prominent here and in immediately adjoining regions than at any other level. There are no further changes in the cortex or epidermis, except increase in length. The length of each cell eventually becomes eight to ten times its width. Ultimately both are abraded. This loss begins early and proceeds gradually and irregularly. Apparently no cortical periderm is formed. Study of mature roots indicates that a periderm is formed by the pericycle as it becomes exposed by the final disappearance of the cortex.

Figure 3 shows a section still higher up, about 10.5 mm. from the tip and 4.5 mm. from the peg. The primary xylem has been augmented by the maturation of metaxylem elements centripetal to the protoxylem, which shows signs of being crushed out. The metaxylem consists of tracheids and large scalariform and scalariform-reticulate tracheae. There has been considerable addition to the primary phloem by the continued differentiation and maturation of metaphloem. Its elements are somewhat more specialized, sieve tubes and companion cells being present in addition to parenchyma. No crushing of the primary phloem was observed in any seedling material. In this respect, the functional persistence of the primary phloem, the situation is apparently similar to that reported for the

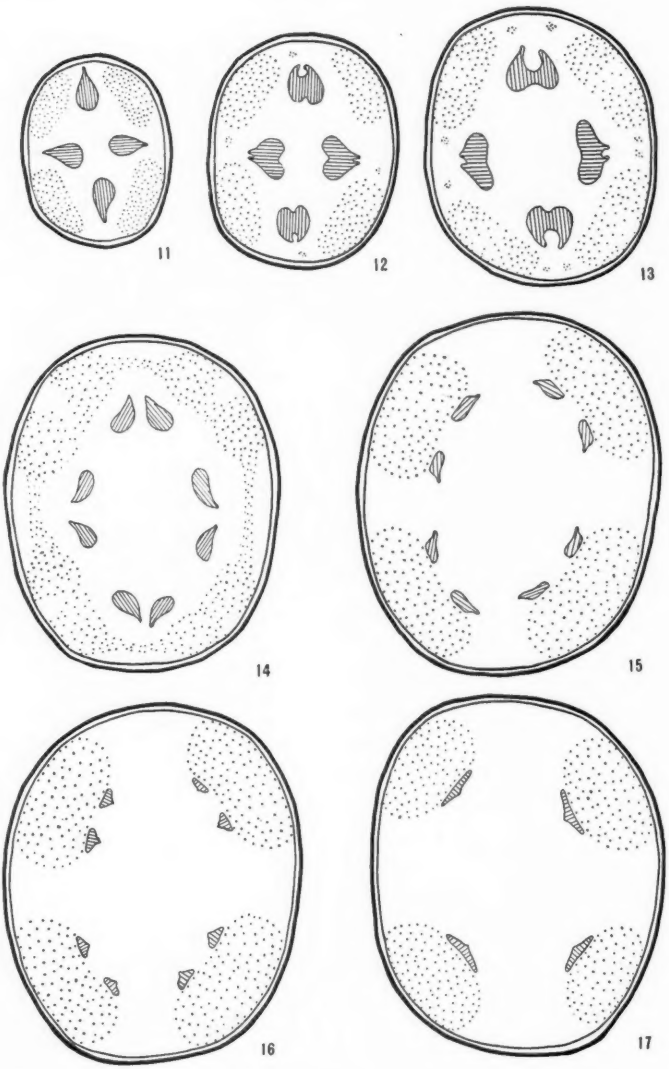


FIGS. 3–10.—Transverse sections from primary root about 15 mm. long showing differentiation of metaxylem and metaphloem and critical stages of transition. Figs. 3–5, stele and endodermis; figs. 6–8, stele, including pericycle; figs. 9, 10, one transition bundle only (*en*, endodermis; *pcl*, pericycle; *px*, protoxylem; *p ph*, protophloem; *mx*, metaxylem; *mph*, metaphloem). ×27.

genus *Cucurbita* (3). The pericycle of this and of succeeding levels is irregularly one to three cells thick, the extra layers being particularly noticeable opposite the protoxylem. Casparian strips are still prominent. The pith is more extensive than at lower levels, owing more to the increase in size of its cells than to their increase in number. The pith is present throughout the length of the primary axis. The combined proliferations of the various tissues and the growth of cells have practically doubled the width of the stele at this level as compared with the one just described. This rapid flaring of the primary root continues up to its mergence with the hypocotyl at the level of the peg.

Figure 5 represents a section 2.5 mm. above the one just described. The most notable change in the primary xylem is the much greater width of each of the four groups, due to continued differentiation of metaxylem laterally. There is evidence that each xylem group is about to diverge radially into two groups. The phloem groups are also much wider, owing to the greater number of parenchymatous cells between the smaller units within each large group. Small clusters of phloem elements are also sparsely scattered in the rays opposite the xylem. Sieve tubes and companion cells are well differentiated in the phloem at this level. The Casparian strips are not so distinct, being most prominent opposite the phloem.

Beginning here also there is rapid reorientation of the vascular bundles from the exarch and radial alignment of the root to the endarch and collateral alignment of the stem (figs. 11–17). First there is a radial separation of each xylem group to form two groups. These new groups apparently are diverged with the metaxylem ends turned away from each other and swung centrifugally through arcs of 180°. At about the time the protoxylem is lateral in position, the two sub-groups of phloem within each large group are far enough apart exactly to subtend the reoriented groups of xylem. This divergence of the phloem is sufficient to separate, almost if not quite, each phloem group into two distinct groups. At this time there are apparently eight bundles, therefore, but these are evidently in four closely aligned pairs. The continued lateral development of the xylem groups results in the members of each of the four pairs coming together to form four endarch collateral bundles, com-



FIGS. 11–17.—Diagrammatic drawings, based on figs. 3–10, showing nature of transition. Outer heavy line represents endodermis; inner line, pericycle; dotted areas, primary phloem; and lined areas, primary xylem.

pleting transition. Finally there are as many bundles as at the beginning. The phloem of each new bundle is that of one of the original groups, while the xylem is related to two bundles, half of each being continuous with the lateral half of two of the original bundles. These two strands maintain their entity for a time, but there is less parenchyma differentiated between them so that there is soon but one compact group. This final alignment of the four endarch bundles occurs at the lower level of the peg, scarcely 2 mm. above the level of the beginning of transition.

There are other important histological situations in the transition zone which are not particularly related to transition itself. These are indicated in the diagrams and in the detailed drawings made of sections taken at critical levels. Figures 12–14 show the presence of phloem across the rays opposite the xylem, while figures 15–17, taken at higher levels, do not show it. Figures 3–10 are of corresponding portions, as indicated in the diagrams. More and more sieve tubes and companion cells, separated by small groups of parenchyma, are differentiated, so that eventually there are no rays, but a continuous band of phloem. This is of considerable physiological importance, since it gives every portion of the root a connection with food channels so long as even one bundle retains its food conducting ability. A second important fact is that the tissues in the upper transition regions (at peg level) are more juvenile or nearer the embryonic condition than are those farther down the axis. This is particularly noticeable in the relative maturity of the xylem elements. This retention of meristematic activity is related to the rapid growth of the lower portions of the hypocotyl, especially during germination.

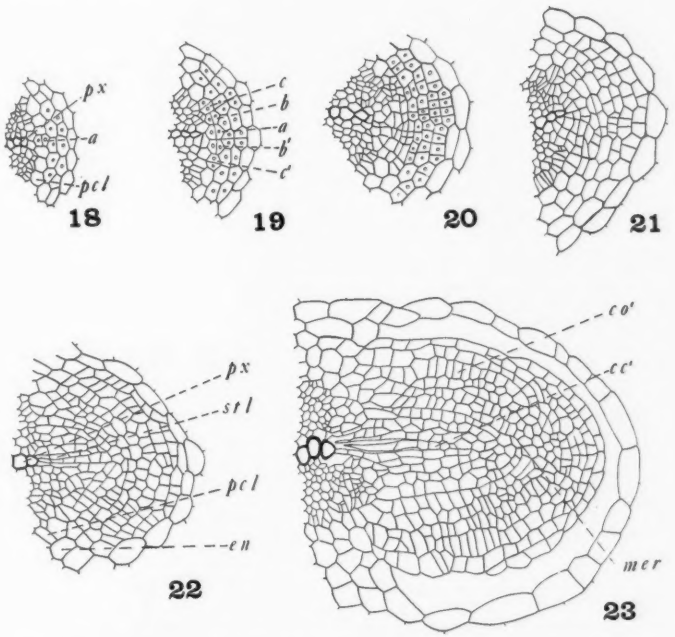
Origin of secondary roots

In addition to the abundance of secondary roots, they are characterized by their very early appearance from the viewpoint of ontogeny, and by the unusual manner of their origin. In general, the manner of origin agrees with that described by Janczewski (**6**). Le Maire (**8**) confirmed the work of Janczewski and elaborated somewhat on the mechanics of development in the different types of origin of secondary roots. Both considered this particular type

to be peculiar to the cucurbits and the legumes; both agreed as to the general plan of development; and both found considerable difficulty in tracing the exact procedure because of the tardy differentiation of primary tissues in the secondary roots. They concluded that the origin and nature of the meristem of the secondary root is as unusual as that of the primary root. Briefly they characterized this type as follows. Only the central cylinder of the secondary root arises from the pericycle of the primary; the remaining tissues are derived from the endodermis and the inner layers of the cortex.

Figures 18–23 are from cross sections of the primary root taken from levels successively back from the apical meristem, and are selected to show the activities of various tissues of the primary root in the development of secondary roots. Figure 18 shows a single cell of the pericycle which has divided tangentially. It is exactly opposite a protoxylem point. At the time this division occurs the protoplasm is dense within the adjoining cells of the pericycle, as well as in the surrounding cells of the endodermis and proximal cells of the immediately adjoining cells of the cortex. Figure 19 shows cells on either side of the initial divided, also tangentially, while the one next to each of these has divided radially. These two cells, which divide radially, mark the limits of the pericycle which enters into the formation of the secondary root. In addition several cells of the endodermis have divided tangentially, and some of the cells so derived have divided radially, thus outlining the beginnings of the secondary root in the cortex of the primary. Likewise radial divisions have occurred in the two or three layers of pericyclic cells previously mentioned as occurring opposite the xylem points. JANCZEWSKI (6) refers to these inner cells, together with those of the main layer of the pericycle, as pericambial, because of their relation to the formation of secondary roots. Figure 20 shows continued proliferations of all the tissues and also divisions beginning to occur in an adjacent layer of the cortex. Figure 21 shows, in addition to continued divisions, the beginning of demarcation of the central cylinder of the lateral root. In figure 22 this demarcation is still more definite. The stele of the secondary root is also accentuated by cells which form the xylem connections between the secondary root and the

xylem strand opposite which it is formed. Here too is evidence of the formation of the terminal meristem of the lateral root. Because



FIGS. 18–23.—Cross sections from primary root showing development of secondary roots: Fig. 18, single cell (*a*) of pericycle divided transversely to become a secondary root initial; other nucleated cells are results of divisions of endodermal cells (*px*, protoxylem; *pcl*, pericycle). Fig. 19, two pericyclic cells (*b*, *b'*) adjoining the initial have divided tangentially and two cells (*c*, *c'*) adjoining these have divided radially; all other nucleated cells still of endodermal origin. Figs. 20–21, continued development of secondary root tissues through proliferations of pericyclic and endodermal tissues. Fig. 22, stele of secondary taking form and inner cortical layers of primary contributing to outer tissues of secondary (*pcl*, pericycle of primary; *en*, endodermis of primary; *co*, cortex of primary; *stl*, steler tissues of secondary). Fig. 23, secondary ready to emerge through last cortical layer of primary (*cc'*, central cylinder of secondary, developed from pericycle of primary; *co'*, cortex of secondary, developed from endodermis and inner cortex of primary; *mer*, developing meristem of secondary). ×35.

of the extent to which various tissues of the primary root have contributed to the formation of the secondary, it is difficult to recognize

with exactness the origin of the terminal meristem of the latter. JANCZEWSKI assumed that finally it is derived partly from the terminal cells of the central cylinder and partly from the cells, of endodermal origin, which surround the tip of the central cylinder. I was unable to determine from the material studied the exact origin of the cells in this meristematic region. From such as were examined, as illustrated in figures 22–23, such an opinion seems valid. Apparently a considerable portion of this tissue is of endodermal, and perhaps cortical, origin. Figure 23 shows the lateral root just ready to emerge from the primary. It shows the extent to which the endodermis and cortex have contributed to the building up of the tissues of the secondary roots. There is little or no solution and resorption of the primary cortex as the secondary root penetrates it. Subsequent derivation of cells of the lateral root is from its meristem, which, according to JANCZEWSKI, functions similarly to that of the primary root.

PEG

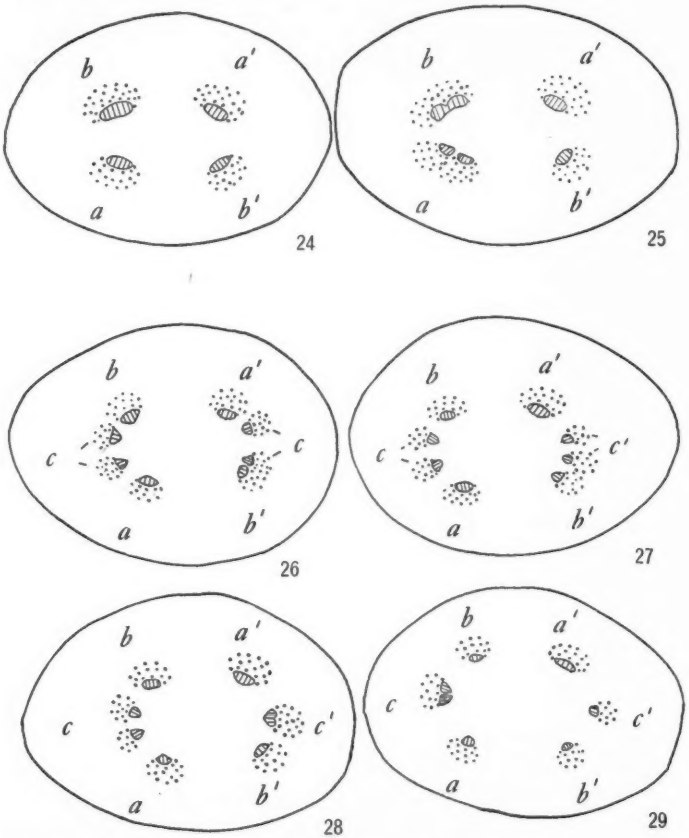
The peg is located on the axis exactly at the level of transition. Spinulose epidermal hairs, characteristic of the hypocotyl, occur on the upper face of the peg, and root hairs on its lower face. Since the peg has been adequately described by CROCKER and others (2) for other genera of the Cucurbitaceae, and since no variation from their findings was found in *Citrullus*, no detailed discussion is deemed necessary here. The most pronounced development of a peg occurs when seeds are germinated in a flat position. In such position the cortical cells composing the peg have their longest diameter in a radial plane, whereas elsewhere their longest diameter is in the vertical plane. There are also more layers of cells across the cortex at the peg than are found elsewhere in it. That it may appear at any radius or on opposite sides, in the case of seeds planted in an erect position, indicates that the peg is a localized cortical structure developed in accordance with the direction of the stimuli which initiate it.

HYPOCOTYL

As previously stated, the hypocotyl is flattened in the plane of the cotyledons. At its lowest level, about where transition is com-

pleted, there are four transition bundles. These are not uniformly spaced but are paired, with more space between the pairs than between the two bundles of a pair. This spacing of the bundles is associated with the flattening of the hypocotyl. Following transition, divergences occur in all, or at least in two, of the four bundles. Usually branches diverge from each of the two bundles which are paired toward the ends of the oval as seen in cross section. These branch bundles always come from the sides of the bundles facing each other, so that they are at the ends of the oval. They move together rapidly, and anastomose at levels varying from 1 to 5 mm. above the peg. There is some variation. Sometimes only one of each of the paired bundles branches and sometimes two of one pair may branch and only one of the other pair. In most cases both of the pairs branch. In all cases, when the process has been completed there are six bundles, now so spaced as to appear as two sets of three at opposite ends of the oval. Figures 24–29 are of transverse sections taken from successively higher levels in this zone, and show how the six bundles of the hypocotyl arise from the four transition bundles. Figure 30 is a schematic drawing made from cleared specimens showing the same features and also the xylem in transition. In many cases the bundles of the hypocotyl continue as six up to the cotyledons. In other cases additional branching may increase the number to eight. These two additional branches are also divergences from one or more of the original transition bundles. They are always from the side of the bundle opposite that from which the first branching occurred, and lie along the sides of the oval as seen in cross section. Apparently these two new bundles may come from any of the four but they are always on opposite sides of the hypocotyl, evidently providing food channels through the extensive tissues lying between the sets of bundles already present. Moreover there is no fixed level at which they diverge. Sometimes one or both may appear near the base of the hypocotyl; more commonly they appear higher up. In the region just below the cotyledons, the branch bundles lying along the flattened sides of the hypocotyl, provided such branches have formed, anastomose with one of the parent bundles between which they lie. Usually such a branch is reunited with the bundle from which it is diverged. In a few cases

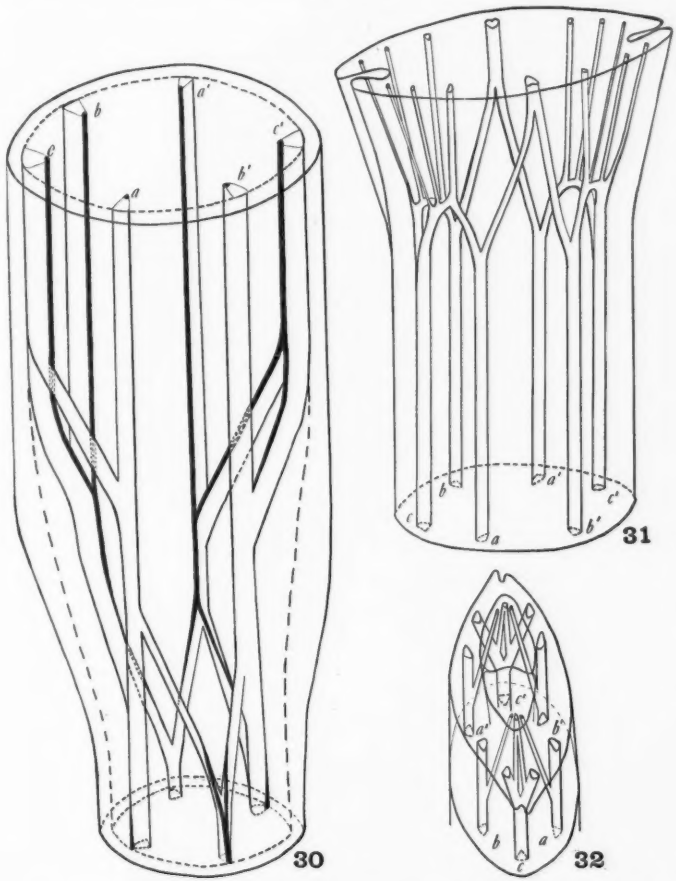
it is separated into two strands, one going to each of the two bundles between which it lies. In all cases observed this anastomosing again reduces the number to six, at or below the level at which they



FIGS. 24–29.—Diagrammatic drawings of cross sections from lower hypocotyl showing origin of six bundles of hypocotyl from four transition bundles. *a*, *a'* and *b*, *b'* are opposite transition bundles; *c* is formed of anastomosing branches from *a* and *b*, while *c'* is likewise developed from *a'* and *b'*.

enter the cotyledons. Because very often there may be no more than six bundles in the hypocotyl, and because the additional bundles, if present, are irregular as to the levels at which they occur and

always anastomose with the parent bundles, six bundles are considered the typical number, as shown in all illustrations. The



FIGS. 30–32.—Schematic drawings showing general plan of vascular system: Fig. 30, root stem transition (below) and organization of six bundles of hypocotyl from four transition bundles (above). Fig. 31, manner in which hypocotyledonary bundles pass into cotyledons. Fig. 32, vascular connections to epicotyl. Bundles lettered throughout as in figs. 24–29.

bundles are bicollateral throughout the length of the hypocotyl. There is considerably more external phloem than xylem, so that it

extends far around the xylem group in each bundle. The rapid differentiation of phloem soon provides a striking preponderance of phloem. The various elements within the bundle are similar to those of the root. Figures 47–49 show these features of typical hypocotyledonary bundles. There is no interfascicular cambium. Consequently all thickening, aside from the small amount accomplished by fascicular cambium, is owing to activity of the parenchymatous tissues. This feature was reported by POTTER (9).

The epidermis is composed of greatly elongated and flattened cells. It is richly supplied with stomates.

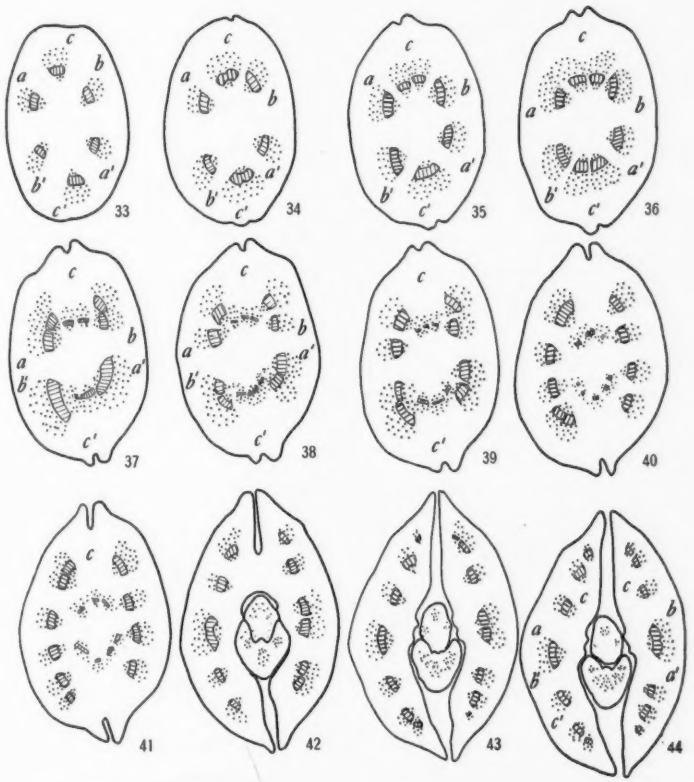
The cortex is like that of the root except that the cells become even longer, and are filled with chloroplasts to considerable depth. The endodermis and pericycle are irregular and indistinguishable as a rule. It is possible to trace the pericycle.

The central pith begins to disintegrate early throughout the length of the hypocotyl. Such disintegration is limited to the larger cells in the central region; the outer portions of the pith remain intact and are parenchymatous, especially in the region of the bundles.

COTYLEDONS

All bundles of the hypocotyl terminate in the cotyledons. There are five main bundles at the base of each cotyledon, the three centrally located ones being very prominent. In addition to the five there is a smaller branch bundle at each edge of the cotyledon. These seven form the primary framework of the whole structure. This bundle alignment is derived from the six bundle arrangement of the hypocotyl (figs. 33–44, 31). The tapering of the hypocotyl toward the top amounts almost to a constriction at the level of divergence of the cotyledons. In this region the three bundles located in each of the two ends of the oval, as seen in the cross section, are so close together as to form apparently one large bundle, in which, however, the three bundles maintain their identity. A cross section at this level (fig. 36) shows these two semicircular bundle groups with prominent rays between. At the same time the two bundles in the ends of the oval, the last formed of the six hypocotyledonary bundles, begin to divide into two separate

bundles. Immediately these, and likewise the other bundles, move outward from the semicircles toward the rays. When the separation is complete there are four bundles along each side of the oval. The two inner ones on each side are the original four transition



FIGS. 33–44. Transverse sections from upper hypocotyl showing divergence of hypocotyledonary bundles into cotyledons and vascular connections to epicotyl.

bundles, by position at least, while the two outer ones are branches from the bundles lying in the ends of the oval and formed by the anastomosing of strands from the original four. The two inner bundles of the four on each side continue to move toward each other

and finally unite to form the midribs of the two cotyledons. The outer two bundles of each cotyledonary group branch and thus increase the number to five, and this number is soon increased to seven by the smaller branches which again diverge from the outermost bundles. All the bundles of the cotyledons are loosely joined by archlike connections (fig. 31) which are produced in the apparent moving together of the hypocotyledonary bundles to form the semicircles. For the most part, these connections are composed of phloem tissue.

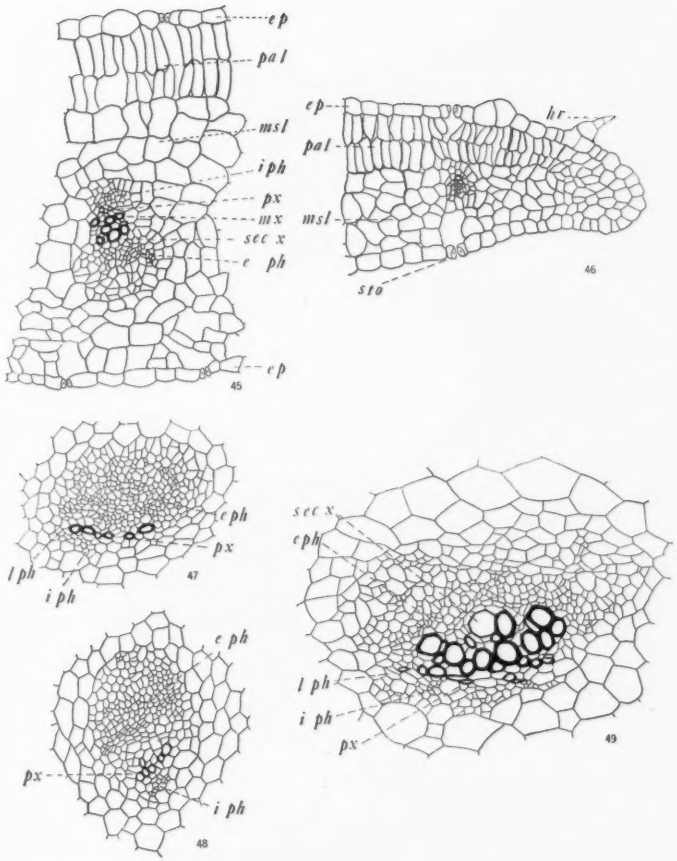
Simultaneously with establishment of their vascular framework, the cotyledons also diverge from the axis. The epidermal cells are very irregular in shape and size, presenting a mosaic-like surface view characteristic of many dicotyledonous plants. Stomata are numerous in both the lower and the upper surfaces. Their long axes lie in all directions. The hairs of the upper epidermis are simple, composed of two to five cells pyramided on a large basal cell which is surrounded by a cordon of large epidermal cells.

There are two layers of elongated cells in the palisade, which is rather loosely arranged with considerable air space, especially near the stomata. The cells of the mesophyll are more or less isodiametric, and arranged so as to provide for large air spaces in the vicinity of the stomata of the lower epidermis. They are compact around the bundles, forming a rather thick supporting sheath.

The bundles form an extensive network of veinlets through the mesophyll. They are bicollateral and undergo considerable secondary thickening. Figures 45–46 show transverse sections from the cotyledons, selected so as to show the general features described.

Epicotyl

No extensive studies of the epicotyl were made other than to establish the nature of its vascular connections to the hypocotyl (fig. 32). There are usually six bundles in the epicotyl at the level where its first leaf diverges. These six bundles are branches from the six bundles of the hypocotyl, and have the same relative position with respect to one another and to the axis. In cross section they appear as two sets with three bundles in each set. One set of three enters the first leaf and the other set enters the second leaf. Branch-



FIGS. 45–49.—Figs. 45, 46, transverse sections of cotyledons: fig. 45 from central portion and fig. 46 at margin (*ep*, epidermis; *pal*, palisade; *msl*, mesophyll, *i ph*, internal phloem; *px*, protoxylem; *mx*, metaxylem; *sec x*, secondary xylem; *e ph*, external phloem; *sto*, stomate; *hr*, epidermal hair). ×30. Figs. 47–49, transverse sections of hypocotyledonary bundles showing origin of internal phloem: Fig. 47, transition bundle, at peg level, of five days old seedling (*l ph*, lateral phloem; *pi*, pith). Fig. 48 (from level just below cotyledon, same aged plant), internal phloem forming by divisions of pith cells. Fig. 49, same position as fig. 47 but of a nine days old seedling. ×27.

ing of these six bundles of the epicotyl from the bundles of the hypocotyl occurs in the following manner. At or just below the level at which the cotyledons diverge from the axis, branch bundles to the epicotyl diverge from each of the four transition bundles. At this level the two remaining bundles of the hypocotyl have already branched preparatory to entering the cotyledons. Divergences to the epicotyl occur from each of these branches, and soon anastomose to form the bundles of the midribs of the first and second leaves of the epicotyl respectively. Cases occur in which these bundles of the midribs diverge as single strands from the corresponding bundles of the hypocotyl, before they branch to the cotyledons (figs. 37–41).

The divergences of the bundles to the epicotyl begin at a much earlier stage than that used for illustrative purposes. In fact their development is evident in seedlings whose hypocotyls have hardly emerged from the integuments. In such early stages, however, these nutritive connections between the axis and the epicotyl appear only as two arcs of undifferentiated, highly parenchymatous tissue lying in the zones where the actual vascular connections are to develop. Within these arcs of parenchyma again, the phloem differentiates before the xylem. The bundles are bicollateral from the beginning. It is only when the tissues of these new bundles have been well differentiated that the vascular connections to the epicotyl can clearly be determined.

When these bundles have definitely been established, a zone of tissue differentiates across the epicotyl between the two sets of bundles, and the first leaf soon diverges from the axis (figs. 32, 42–44).

Summary

1. Early stages of development of the watermelon seedling and its gross structure are described.

2. The primary root is tetrarch exarch. In transition each xylem strand is divided into two. These two divisions swing outward through arcs of 180°, and converge by pairs in front of the four phloem groups. When transition is completed there are four bundles composed of the four original phloem groups intact and of xylem which comes in approximately equal amounts from two adjoining

groups of xylem of the original alignment. The transition is rapid and is completed at the level of the peg.

3. There is one common histogen in the primary root; all primary tissues are derived from a common meristematic region.

4. The endodermis and inner cortical layers, as well as the pericycle, of the primary root contribute to the building up of the tissues of the secondary roots.

5. There are usually six bundles in the hypocotyl: the four transition bundles and two others which are anastomosed branches from the transition bundles. All these bundles diverge into the cotyledons.

6. For a considerable period of time the cotyledons serve as photosynthetic organs.

7. The epicotyl develops slowly at first, because its vascular connections with the axis are, in the main, differentiated after germination.

8. The bicollateral bundles extend from the level at which transition is completed upward through the hypocotyl into the cotyledons and into the epicotyl. The internal phloem is derived from the parenchyma which lies adjacent to the inner faces of the bundles. It does not extend into the roots, but is connected laterally with the external phloem in the transition zone.

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EMBRYOLOGY OF PISUM SATIVUM[1]

D. C. COOPER[2]

(WITH THIRTY-ONE FIGURES)

Introduction

Little information concerning the embryology of *Pisum sativum* L. or of any other member of the Vicieae tribe of the Leguminosae is to be found in the literature. Perhaps the earliest reference is by TULASNE (9), who described stages in the development of the embryo of *Lathyrus aphaca* L. He reported the presence of a suspensor of peculiar type, consisting of two long filiform basal cells and two shorter but larger cells closely adjacent to the embryo. HOFMEISTER (6) found that the embryo of *Lathyrus odoratus* L. is pushed to the mid-region of the embryo sac as a result of elongation of the suspensor. STRASBURGER (8) called attention to the multinucleate condition of the suspensor cells in *Orobus*. The most extensive study of the embryology of members of this tribe is that of GUIGNARD (5), who examined twenty-three species, including eight of *Orobus* (*Lathyrus*), six of *Vicia*, two of *Pisum*, and two of *Ervum* (*Vicia*). He described in detail the development of the embryo sac and embryo of *Orobus angustifolius* L. (*Lathyrus canescens* Gren. and Godr.), and indicated that the same type of development is characteristic of other members of the tribe. MARTIN (7) found a similar type of embryo development in *Vicia americana* Muhl.

Material and methods

The material for this investigation was collected from plants growing in the greenhouses and field plots of the Department of Genetics, University of Wisconsin, during the spring and summer of 1937. Two commercial varieties (Little Marvel and Asgrow Pride) of the

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[2] The writer desires to express his appreciation for support received from the Wisconsin Alumni Research Foundation during the period of this investigation.

common garden pea (*Pisum sativum*) were used. Ovaries from late buds and open flowers and pods of various ages were immersed in Carnoy's alcohol-acetic-chloroform mixture for 2–5 minutes, and then transferred to Karpechenko's modification of Navashin's fluid. The older pods were opened and the young seeds removed and similarly treated. After imbedding in paraffin, sections were cut from 12 to 20 μ in thickness (depending upon the age of the material), mounted serially, and stained in dilute Delafield's haematoxylin. No significant differences were found between the two varieties studied. The description which follows is based chiefly upon Asgrow Pride.

Observations

POLLINATION AND FERTILIZATION.—Each ovary contains from eight to ten campylotropous ovules arranged alternately along the ventral suture. The curvature of each developing ovule is toward the style. At maturity the micropyle is thus brought into close proximity to the ventral suture, the opening of the micropyle facing the basal end of the ovary.

The mature embryo sac just before fertilization is an elongate, seven-celled, eight-nucleate structure. The curvature of the ovule has caused the embryo sac to be bent at an obtuse angle in the mid-region. The three irregularly shaped antipodal cells at the chalazal end of the sac are very small and disintegrate shortly after fertilization.

The egg apparatus consists of three pear-shaped cells. The egg, which is much larger than either synergid, contains a large nucleus imbedded in the dense cytoplasm in its basal region and a large vacuole at the micropylar end. A large vacuole occupies the enlarged basal portion of each synergid, and a series of elongate vacuoles or canals extends forward from this region toward the apex of the cell. The nucleus lies in the dense cytoplasm near the mid-portion of the cell, just above the basal vacuole.

The two polar nuclei are closely appressed. They lie near the bend of the embryo sac in a mass of denser cytoplasm which extends from the egg apparatus toward the chalaza.

Pollination takes place approximately 24 hours before the open flower stage is reached. The standard at this time is greenish white

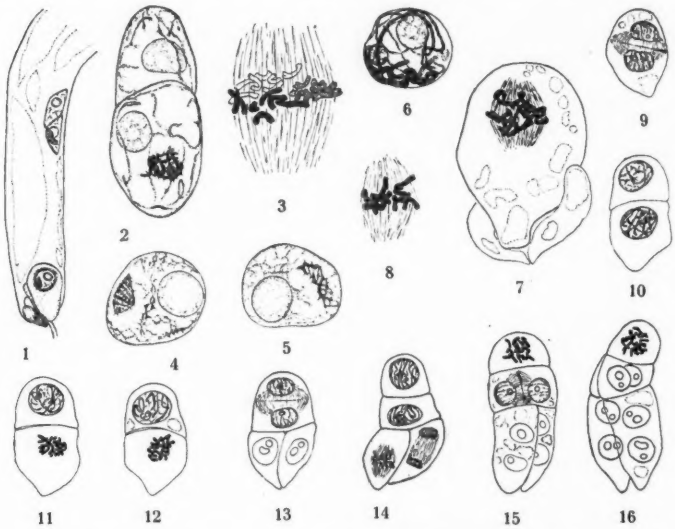
in color; the wings more or less tightly inclose the keel. Fertilization has taken place, and either the zygote is in the process of division or a two-celled proembryo is present at the time the flower is fully expanded. The pollen tubes grow along the ventral suture of the ovary, and when they reach the micropyle of the ovule one or more attempt to enter it. One pollen tube grows into the micropyle and enters the embryo sac between the apices of the synergids and egg (fig. 1). Although in some instances many pollen tubes were observed at the entrance of the micropyle, no cases were observed in which more than one had entered the embryo sac. The synergids are not broken down by the pollen tube as in *Phaseolus vulgaris* (10), but persist for a short time after fertilization, as in *Melilotus* and *Medicago* (2, 3).

The two male gametes are discharged from the pollen tube into the embryo sac in the vicinity of the egg. Each male gamete, as found in the pollen tube, is surrounded by a definite layer of cytoplasm (4). Whether the cytoplasmic sheaths are shed when the gametes leave the pollen tube or after they have entered the embryo sac was not determined. One male gamete nucleus becomes closely appressed to the egg nucleus in the process of fertilization; the other becomes appressed to one of the polar nuclei (fig. 1). Each male nucleus is somewhat elongated with a nucleolus near one end as in *Medicago* (3) and not rounded as in *Phaseolus* (1).

The small densely staining male gamete nucleus, which is appressed to one of the polar nuclei, increases in size, the nuclear membranes disappear along the surface of contact, and the chromatic network of the male nucleus spreads out but never as extensively as does that of either polar nucleus (fig. 2). The three nuclei of this group divide simultaneously, and the groups of chromosomes are brought on to a common spindle (fig. 3). The three chromosome groups may be identified in figures prior to and including the equatorial plate stage.

The second male nucleus becomes closely associated with the egg nucleus, and the nuclear membranes disappear in the region of contact (fig. 4). The chromatic network of the male nucleus spreads out for a time (fig. 5), and then condenses in advance of that of the egg nucleus. As a result, short well developed chromosomes appear

in one portion of the fusion nucleus, whereas the remainder of the nucleus contains elongated chromatic threads in an early prophase condition (fig. 6). The nucleolus brought in by the male nucleus



FIGS. 1–16.—Fig. 1, micropylar portion of embryo sac showing double fertilization. Fig. 2, male gamete nucleus fusing with polar nuclei. Fig. 3, first division of primary endosperm nucleus showing three separate groups of chromosomes. Fig. 4, early stage in process of fusion of male gamete nucleus with egg nucleus. Fig. 5, somewhat later stage; limits of male gamete nucleus still clearly defined. Fig. 6, zygote nucleus in prophase; chromosomes from egg nucleus less condensed than those from male nucleus. Fig. 7, egg apparatus; zygote with lateral view of equatorial plate; chromosomes at right side of spindle on plate, those on left side being drawn to plate. Fig. 8, lateral view of zygote equatorial plate showing two groups of chromosomes. Fig. 9, binucleate proembryo showing cell plate formation. Fig. 10, two-celled proembryo. Figs. 11, 12, two-celled proembryos; nuclei of basal cells in division; nuclei of apical cells in prophase. Fig. 13, three-celled proembryo; cell plate formation in apical cell. Fig. 14, four-celled proembryo; nuclei of two basal cells in division. Fig. 15, same; basal cells binucleate; cell plate formation in middle cell; nucleus of apical cell dividing. Fig. 16, five-celled proembryo; two binucleate basal cells, two uninucleate middle cells; nucleus of apical cell dividing. Fig. 1, ×185; figs. 2–8, ×880; figs. 9–16, ×450.

enlarges to some extent but never approaches the size of the nucleolus of the egg.

Two distinct groups of chromosomes can be recognized on the

mitotic spindle in the zygote (figs. 7, 8). The chromosomes on the right side of the spindle shown in figure 7 are in a plane above those on the left side. In the plate shown in figure 8, likewise, there is a definite plane of separation between the two groups. The details of gametic fusion and the division of the zygote nucleus are very similar to those previously described for *Medicago* (3).

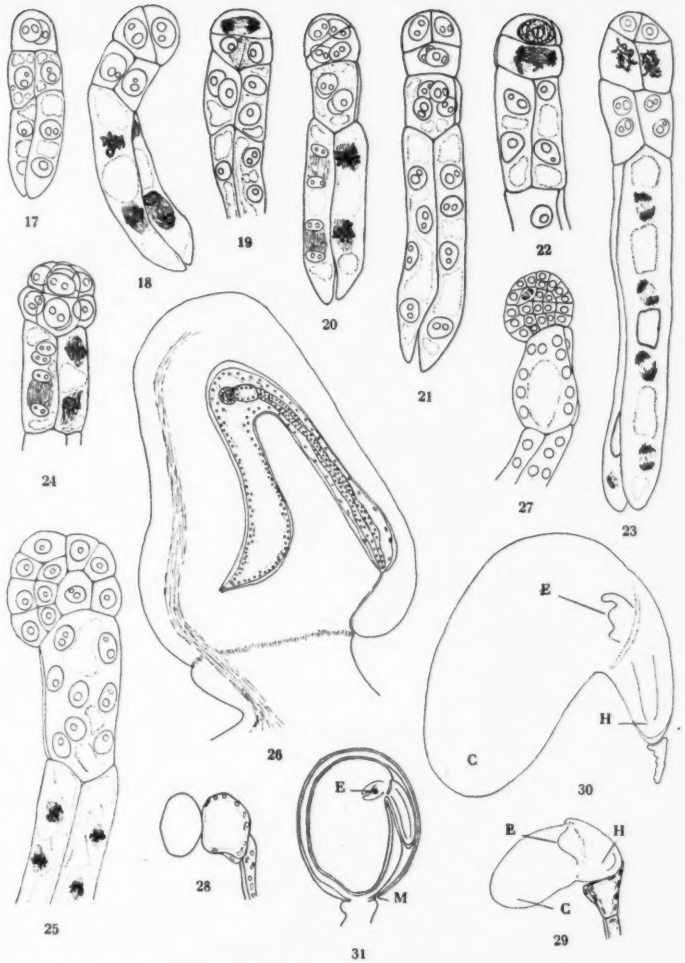
ENDOSPERM.—One of the two nuclei formed as a result of the division of the triple fusion nucleus migrates toward the chalazal end of the embryo sac and the other to a position near the zygote. Shortly thereafter a second division occurs and the four resulting nuclei take peripheral positions in the dense cytoplasm surrounding the large central vacuole. The endosperm possesses usually two, sometimes four, nuclei before completion of the division of the zygote nucleus. This is similar to the condition found in *Melilotus* and *Medicago* (2, 3). GUIGNARD (5) found that the zygote of *Orobus angustifolius* divides prior to the first division of the primary endosperm nucleus, and MARTIN (7) found that the divisions of zygote and primary endosperm nuclei are simultaneous in *Vicia americana*. No instances have been found in the material of *Pisum* thus far examined of a division of the zygote nucleus before division of the primary endosperm nucleus.

Divisions of endosperm nuclei continue in rapid sequence. The early divisions are simultaneous, but at later stages of development this may not be the case. The dividing nuclei in any particular area of the endosperm, however, are in approximately the same stage of mitosis. Thus the divisions of those nuclei in the region of the embryo may be at metaphase and those in the chalazal region in a prophase stage.

The endosperm is multinucleate at stages such as that shown in figure 26. Nuclei are present in greater numbers in the dense cytoplasm immediately surrounding the embryo than elsewhere in the endosperm. With the exception of the region immediately adjacent to the hypocotyl and extending from there to the micropyle, the endosperm remains multinucleate throughout its development. Cell formation is initiated in the neighborhood of the hypocotyl and of the basal portions of the cotyledons in ovules in which the embryo has reached the stage shown in figure 29. It continues toward the

micropyle so that all the endosperm in this region becomes cellular. According to GUIGNARD, cell formation is totally absent in the endosperm of members of the Vicieae. The endosperm of *Pisum* is resorbed in the course of embryo development, and there is little if any evidence of it in the mature seed.

EMBRYO.—The zygote divides transversely (fig. 9) to form a two-celled proembryo, the two cells being approximately equal in size (fig. 10). The basal cell (that is, the one nearer the micropyle) now divides longitudinally to form two suspensor cells (figs. 11–13). Shortly thereafter the apical cell divides transversely, forming an apical embryo mother cell and a middle cell (figs. 13, 14) which in the course of development is to form a bulbous middle piece. The nuclei of the two basal suspensor cells divide (fig. 14), and, cytokinesis not following, binucleate cells are formed (fig. 15). Next occurs a longitudinal division of the middle cell, the plane of division being at right angles to that which occurred to produce the two basal cells. Shortly thereafter the nucleus of the apical embryo mother cell divides, the long axis of the division spindle being at right angles to the plane of division of the original middle cell (figs. 15–17). The plane of cytokinesis in the apical cell is at an oblique angle (fig. 18). The basal cells have been elongating during this period of development, and their nuclei as well as those of the middle cells divide, producing respectively four-nucleate and binucleate cells. The divisions in the basal cells in some instances precede those in the middle cells (fig. 18); in other cases they follow (fig. 20). The two cells of the embryo proper divide longitudinally, the planes of division being at right angles to each other, to form a four-celled embryo (figs. 19–21). Each of these cells divides again (figs. 22, 23), producing an eight-celled embryo consisting of two layers of four cells each (fig. 24). Nuclear divisions but no cell divisions occur in the suspensor cells, so that each of the basal cells has eight nuclei and each of the middle cells has four nuclei by the time an eight-celled embryo is formed (figs. 23, 24). The cells of the embryo continue to multiply, and an undifferentiated spherical mass of cells is formed (fig. 27). The suspensor in the meantime continues to develop (fig. 25) and reaches its greatest size at a stage such as shown in figure 26. The two basal cells, which have elongated and now



FIGS. 17–31.—Fig. 17, two-celled embryo, four-celled suspensor; ×450. Fig. 18, same; two nuclei of each basal cell dividing; ×450. Fig. 19, embryo showing divisions leading to four-celled stage; ×450. Figs. 20, 21, four-celled embryo and suspensor; basal cells of suspensor have four nuclei; middle cells binucleate; ×450. Figs. 22–24, divisions in embryo leading to eight-celled stage; fig. 23 shows dividing nuclei in basal cells and fig. 24 similar divisions in middle cells; ×450. Fig. 25, somewhat later stage; middle cells eight-nucleate; nuclei of basal cells dividing; ×450. Fig. 26, longitudinal section of young seed showing greatest development of suspensor; elongating basal cells have pushed middle piece and embryo to region of bend of ovule; ×45. Fig. 27, embryo at slightly later stage; ×185. Fig. 28, embryo somewhat broadened and flattened; nuclei of suspensor cells in early stages of disintegration; ×45. Fig. 29, early differentiation of parts of embryo: *C*, cotyledon; *E*, epicotyl; *H*, hypocotyl; ×23. Fig. 30, older embryo showing bend in region of epicotyl; ×23. Fig. 31, longitudinal section through mature seed showing relation of parts: *M*, micropyle; ×3.

possess sixty-four nuclei each, have pushed the embryo and the middle cells into the multinucleate endosperm so that they lie in the region of the bend of the ovule. The middle cells have meanwhile grown to form a bulbous middle piece between the basal cells and the embryo. Each of the middle cells has thirty-two nuclei, imbedded in the peripheral layer of dense cytoplasm. A large vacuole occupies the central region of each middle cell. The bulbous middle piece is at this stage fully as large as, if not larger than, the embryo proper. The nuclei of the cells of the suspensor do not divide further. The cells, especially those of the middle piece, may grow to some extent (fig. 28).

With continued development, the embryo broadens and flattens, and that portion adjacent to the middle piece becomes rounded (fig. 28). Certain cell groups on opposite sides of the periphery of the apex, at the center of the apex, and at the base of the embryo become actively meristematic (fig. 29) and the cotyledons (*C*), epicotyl (*E*), and hypocotyl (*H*) are initiated. In figure 29 one cotyledon is removed to show the epicotyl. The nuclei of the suspensor show early signs of disintegration at stages such as that of figure 28, and there is definite distortion of these cells shortly thereafter (fig. 29). The suspensor from this time on rapidly disintegrates, and by the time the embryo has reached the stage of development shown in figure 30 it is in an advanced stage of disorganization. No definite remains of a suspensor could be identified in the mature seed.

The cotyledons and hypocotyl elongate, the former toward the chalaza and the latter toward the micropyle. The embryo becomes curved in the region of the epicotyl to conform to the shape of the ovule (fig. 30). The cotyledons develop at the expense of the endosperm, becoming large rounded structures which occupy most of the space in the chalazal end of the ovule. They act as storage structures, and the endosperm is completely assimilated. The hypocotyl likewise elongates but to a lesser extent, so that at maturity its tip lies approximately halfway between the micropyle and the epicotyl. The longitudinal axes of the hypocotyl and of the cotyledons are approximately parallel in the mature seed. Primordia of the first secondary leaves develop just below the apical meristematic tip of the epicotyl. Figure 31 represents a longitudinal section

through a mature seed. The relationships existing between the various parts just described are shown in this figure. The course of embryo development in *Pisum* is essentially similar to that described by GUIGNARD for *Orobus angustifolius*.

Summary

1. Pollination takes place in *Pisum sativum* between 24 and 36 hours before the open flower stage. Fertilization has occurred and division of the zygote is under way by the time the flower is fully opened.

2. The pollen tube enters the embryo sac between the synergids, neither of which disintegrates until later.

3. One male gamete nucleus fuses with the two polar nuclei. In the division of the primary endosperm nucleus three separate groups of chromosomes are recognizable on the equatorial plate.

4. The primary endosperm nucleus divides before the zygote nucleus. Free nuclear division occurs in the endosperm in the early stages of development. Cell formation later takes place in the micropylar portion of the endosperm, the chalazal portion remaining multinucleate. The endosperm is resorbed in the course of embryo development.

5. During the division of the zygote nucleus, the chromosomes from the two gamete nuclei remain in more or less distinct groups until the equatorial plate stage.

6. The zygote divides transversely to form a two-celled proembryo. The basal (micropylar) cell then divides longitudinally to form two suspensor cells, and the apical cell divides transversely to form an apical embryo mother cell and a middle cell. The middle cell divides longitudinally to form a two-celled middle piece. The nuclei of the two basal and two middle cells divide to form multinucleate suspensor cells having respectively sixty-four and thirty-two nuclei each.

7. The embryo develops in a typical manner from the apical embryo mother cell.

8. The two multinucleate basal cells elongate and push the embryo and middle piece to the region of the bend of the campylotropous ovule.

9. The suspensor cells disintegrate shortly after differentiation of the cotyledons, epicotyl, and hypocotyl.

10. The cotyledons act as storage organs in the mature seed, the endosperm being completely assimilated in the course of embryo development.

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AUXIN DISTRIBUTION IN SEEDLINGS AND ITS BEARING ON THE PROBLEM OF BUD INHIBITION

J. VAN OVERBEEK

(WITH TEN FIGURES)

Introduction

The question of auxin distribution throughout the plant is obviously of importance for the understanding of growth. Of the plants commonly used for the study of growth phenomena, the *Avena* seedling is the only one that has been satisfactorily analyzed in this respect (**14**).

Recently *Zea* and *Pisum* have become important laboratory plants, which has made it necessary to know more about their auxin distribution. Maize is used in these laboratories for the study of the physiology of dwarf plants (**19, 24**) and for investigations on ageotropic corn (**21**). The pea is used for the pea test (**32, 33**), for root formation (**31**), for grafting (**29**), bud inhibition (**12, 16, 17**), and various other experiments.

In this paper an attempt is made (1) to analyze the auxin distribution throughout etiolated maize and pea seedlings; (2) to see how far the distribution of growth in maize seedlings can be explained on the basis of auxin distribution; (3) to study, in pea seedlings, the changes in auxin content and distribution resulting from removal of the terminal bud and subsequent development of a lateral bud—in this connection it was also of interest to know how the auxin distribution is changed in decapitated pea plants to which auxin was applied; (4) to see how the data obtained under (3) fit in with the existing theories on bud inhibition. Part I of this paper deals with (1) and (2); part II with the problem of bud inhibition.

Method

THIMANN (**14**) was the first to study auxin distribution in plants. He found that in *Avena* seedlings the auxin content decreased from the tip to the base of the coleoptile. This was determined by ex-

tracting auxin with chloroform from crushed and acidified plant material. Other extraction methods have been mentioned in a previous paper (**23**). When tried on maize, none of the methods so far described proved to be satisfactory for the extraction of auxin. The acid commonly used by all methods especially decreased the yield. Table 1 gives the result of one experiment in which the extraction

TABLE 1

EFFECT OF ACID ON EXTRACTION. AUXIN DISTRIBUTION IN OAT SEEDLINGS GROWN IN WATER CULTURE AND IN SAND (NOS. 70802, 70928)

EXTRACTION METHOD	PLANT MATERIAL	AMOUNT OF AUXIN EXTRACTED IN DEGREES OF CURVATURE IN AVENA TEST		
Crushed and boiled with 50 cc. ether and 1 drop of 1 N acetic acid for 1 hour	14 corn seedlings	With acid 5.7 Acid omitted 13.3		
Not crushed, left for 15 hours in 50 cc. ether and 0.5 cc. of 0.1 N acetic acid	50 oat seedlings	Amount of auxin per cm.		
		Roots in water		Roots in sand
	Coleoptile (plus leaf)	Acid	No acid	No acid
	Upper third	7.7	7.7	7.1
	Middle third	2.9	2.7	3.0
	Lower third	8.8	19.9	15.5
	Mesocotyl	.....	.....	.....
	Upper third	.....	.....	5.4
	Middle third	14.0*	11.8*	9.3
	Lower third	.....	.....	10.9

* Since the entire mesocotyl of watergrown plants was only 4 mm., the actual figures obtained in the assay were 5.6 and 4.7.

with and without acid was compared. The explanation of this destructive effect of acid may be found in the high auxin-b content of corn plants, which is unstable with acid, as was found by KÖGL, ERXLEBEN, and HAAGEN SMIT (**4**).

In many of the methods the material is crushed, but this procedure was not followed because a prolonged extraction was found to extract the auxin sufficiently. Furthermore, crushing involves much labor when large amounts of material or many samples are used.

Ether was employed for the extraction in this work because it is easier to separate from plant material and water than is chloroform, and its low boiling point makes it easy to concentrate the extracts by distilling the ether off. The entire procedure is as follows.

a. Ether was purified by shaking with $FeSO_4$, CaO, and some water (amounts used are of minor importance), followed by distillation to recover the ether. Purification is very important and was

TABLE 2

WATER CONTENT AND LENGTH OF SEEDLINGS
(NOS. 71012, 14, 22)

PART OF PLANT ANALYZED	TOTAL LENGTH (CM.)	AMOUNT OF WATER PER PLANT IN MG. PER CM. (IF BUDS, IN MG. PER ENTIRE BUD)
Corn		
Coleoptile	2.1	20.5 (92.0%)
Mesocotyl	6.4	34.5 (93.5)
Leaf	2.0	7.1 (85.5)
Oats		
Coleoptile	2.8	7.7 (93.9)
Mesocotyl	0.3	16.3 (91.8)
Leaf	2.7	3.0 (87.5)
Peas		
Terminal bud		30.14 (85.4)
Upper internode	4.9	24.5 (93.5)
Upper lateral bud		2.29 (88.1)
Middle internode	4.3	31.2 (95.0)
Lower lateral bud		1.65 (88.8)
Lower internode	4.2	35.7 (92.3)

done immediately before an extraction was made. The fact that a sample of ether gives a negative benzidine test (**26**), a test which is considered extremely sensitive for detecting peroxides, does not mean that it is pure enough for use in extracting auxins. Such a sample may completely destroy an indoleacetic acid solution when shaken with it.

b. The plant material was weighed, or simply measured if the weight and water content per unit length of a certain batch of plants was known (table 2), cut into the parts to be analyzed, and placed (uncrushed and not acidified) in Erlenmeyer flasks. Then 25 to 50 cc. of ether was added per gram of plant material. The total amount

was never less than 50 cc. This was left in the refrigerator for about 20 hours at 4° C. If for some reason the material could not be tested after this period of time, it was found advisable to evaporate the extract to dryness (see c and d) and store it in the dry condition rather than leaving it in the ether.

c. The ether was then poured off and the residual plant material rinsed with pure ether. Next the ether extract was concentrated by distilling the ether off (water bath) until about 1 to 2 cc. was left.

d. The residue was taken up in a pipette and dropped carefully on the bottom of a small (5 cc.) vial suspended in a beaker of boiling water. Container and pipette were rinsed with a few cc. of pure ether, which also was carefully evaporated into the vial. Thus the extract was evaporated to complete dryness and collected on the bottom of the vial.

e. A known amount of agar (formerly 3 per cent was used, later 1.5 per cent) was next pipetted on the dry extract. As a minimum amount, 0.5 cc. was used. This was stirred and shaken (the vial still in boiling water) to secure a thorough mixing of auxin and agar. After the vial was removed from the boiling water, it was allowed to stand at room temperature for about three hours before the agar was poured into blocks for the *Avena* test. This procedure proved to be necessary in order to obtain a uniform distribution of the auxin in the agar.

f. The agar was melted again in boiling water and poured into a rectangular brass mold (8 × 10.5 × 1.7 mm.), which was cooled by ice for a rapid gelification of the agar. Next this agar plate was divided into twelve equal blocks, which were ready now to be put on decapitated oat seedlings in order to determine their auxin concentration.

g. The *Avena* test employed has been described by WENT and THIMANN (33). A control with a known amount of indoleacetic acid was run at the same time in order to calculate the obtained values of the extract in gammas (0.001 mg.) indoleacetic acid.

h. If the water content had to be determined, the length of the stems, etc., was measured first; then the fresh weight was determined; next the material was left for twenty-four hours in an electric drying oven at 100° C. and the water content determined.

i. The concentration of the auxin in the plant may be calculated from:

$$\frac{C \times I_{1^\circ} \times V_a}{W} \text{ gammas indoleacetic acid equivalents}$$

per 1000 gm. water contained in the plant. C is the curvature obtained in the *Avena* test when the agar blocks (see f) are analyzed. I_{1° is the concentration of indoleacetic acid (gammas per liter) required to give an increase in curvature of 1° in the *Avena* test. This value, of course, is obtained by calculation from auxin concentrations giving a curvature in the range between 5 and 15°. V_a is the volume of the agar in which the residue is taken up (in cc.). W is the water content (in grams) of the plant material extracted.

It should be noted that in this way the auxin content of the plant material is expressed in terms of indoleacetic acid. In the higher plants auxin of the auxin-a type rather than indoleacetic acid may be expected to be present. The activity of auxin-a, according to KÖGL and co-workers, is about twice that of indoleacetic acid. Throughout this paper most amounts of auxin are expressed in indoleacetic acid equivalents. This provides a unit which is independent of the sensitivity of the test plants, and even of the kind of test plant used, thus making comparable, for instance, values obtained by the regular *Avena* test with such obtained by a *Cephalaria* test carried out in the light (**9**).

Part I: Auxin concentrations in Zea and Avena

DISTRIBUTION OF GROWTH AND OF AUXIN IN ZEA SEEDLINGS

Corn seedlings were grown in moist sand in a physiological darkroom at 24° C. and 90 per cent humidity. The distribution of the growth rates of these seedlings was measured by marking each seedling into 1 mm. segments. This was done by stamping thin parallel ink lines on the coleoptile and mesocotyl with a device described by VAN OVERBEEK and WENT (**25**). After a certain period of time ranging from two hours (fig. 3) to twelve hours (fig. 1), the increase in length of the segments was determined. The growth rate of intact plants (curve *i* of fig. 1) increases gradually from the tip to the base of the coleoptile. In the apical region of the mesocotyl,

which is called first internode by AVERY and co-workers (**2**), the growth rate has its highest value. The lower part of the mesocotyl had stopped growing at the time the measurements were made.

For explaining the growth distribution in etiolated seedlings of grasses, WENT (**27**) devised the so-called two-factor scheme. This scheme (**33**, p. 78) is based on the interaction of two factors both necessary for growth. Auxin, one of the factors, is produced at the tip of the plant; it is transported downward and is used up on its

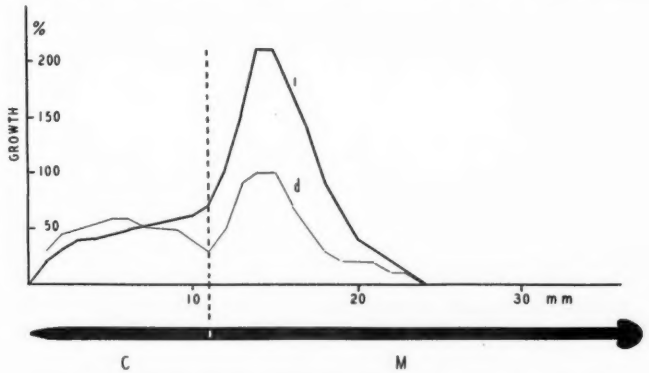


FIG. 1.—Distribution of growth in 4-days-old maize seedlings. Ordinate: percentage growth in 1 mm. segments per 12 hours. Abscissa: location of these segments originally 1 mm. long. *i*, intact plants; *d*, plants from which coleoptile tip was removed 12 hours before final measurements. Average values of 10 uniform plants. No. 60901.

way down. The other factor (or complex of factors) is called the "food factor"; it is supplied by the seed and transported in an upward direction. The food factor too is supposed to be used up while transported. From this consideration it is clear that the growth of the apical regions of the coleoptile is not limited by auxin but by the food factor. On the other hand, the growth of the basal regions of the coleoptile and of the entire mesocotyl is limited by the available amounts of auxin in these regions.

In this connection it was shown by VAN OVERBEEK (**20**) that corn seedlings in which the auxin production was reduced by a heat treatment showed a slight reduction in length in the coleoptile, but the mesocotyl of the treated plants had less than half the length of that

of the controls. If the coleoptile tip of the treated plants was supplied with additional auxin, then the mesocotyl of the treated plants reached the same length as the controls. Similarly it was shown (**19, 24**) that plants in which the auxin is destroyed more rapidly than is normally the case have a mesocotyl which is much shorter than that of normal plants. Such plants are dwarfs. In the dwarf race *nana*, which is shown to have an excessive auxin destruction, the seedlings have coleoptiles which are only a little shorter than those of comparable normal plants; on the other hand, the mesocotyls are several times longer in the normal plants than in the dwarfs. Growth of the mesocotyl does not depend entirely on elongation but also to some extent on cell division (**1**).

How well the two-factor scheme fits in with observations may again be seen from figure 1. Here the auxin production of one set of plants was reduced by removing the upper portion of the coleoptile (about 2 mm.). For a few hours after the decapitation no auxin is produced, but later the auxin production is resumed in the top regions of the coleoptile, but at a reduced rate (**33**). Curve *d* of figure 1 represents the growth curve of such decapitated plants. The growth rate of the upper regions of the coleoptile is slightly increased over that of the intact plants. In the lower regions and in the mesocotyl the growth rate of the decapitated plants is less than that of the intact ones. According to the two-factor scheme this is to be explained as follows. The lower growth rate at the basal regions is due to the reduced auxin production of the decapitated plants because auxin limits the growth of these regions. The increased growth rate of the upper regions of the coleoptile of the decapitated plants is due to the larger supply of food factor in these regions. The basal regions of the decapitated plants have a smaller growth rate than those of the intact ones, hence less food factor is used up in the basal regions of the decapitated plants and consequently more of it will be available for the upper regions of the decapitated plants than for these regions of the intact plants.

The etiolated corn seedling shows a peculiar growth maximum in the upper portion of the mesocotyl, as is shown in figures 1 and 3. For explaining this growth maximum two possibilities may be considered.

1. The maximum may be due to an increase in auxin production in or just above the fast growing region. However, determinations of the auxin content in the various parts of the plant show that the auxin concentration is lowest in the upper portion of the mesocotyl (figs. 3, 4). Any increase in production in this upper portion necessarily must be reflected in an increased auxin content in these regions. Hence this possibility can be safely dismissed.

2. The maximum may be due to an increased sensitivity of the upper portion of the mesocotyl. Evidence that this region is very sensitive to auxin is obtained from figure 2. In this figure plants are shown to which a long narrow strip of auxin paste (lanolin in which indoleacetic acid was dissolved) was smeared unilaterally from the coleoptile tip to the mesocotyl base. After about half an hour the corn seedlings showed the following response: upper part of coleoptile remained straight; basal part of coleoptile slightly bent; apical part of mesocotyl strongly curved; basal part of mesocotyl straight.

Other evidence in the same direction is found in figure 1. In the basal region of the coleoptile the intact plants have elongated 70 per cent over the initial length. The decapitated plants in the same period of time have grown 30 per cent in this region. The ratio of the growth rate intact plants/decapitated plants is 2.3 for this basal region of the coleoptile. The intact plants show a maximal mesocotyl growth of 210 per cent over the initial length. The decapitated plants show a maximal mesocotyl growth of 100 per cent. Hence for the upper portion of the mesocotyl the ratio of the growth rate intact plants/decapitated ones is 2.1. This clearly indicates that the growth reduction due to decapitation is the same in the basal part of the coleoptile as in the upper portion of the mesocotyl. If it were not for the great sensitivity in the upper portion of the mesocotyl, the growth distribution curve would look very much like the one found for *Avena* seedlings grown under ordinary darkroom conditions. Such a curve does not have a maximum as pronounced as the one shown in the curves of figure 1 (see **33**, p. 82). WENT (**27**), studying the mesocotyl growth of *Avena*, also came to the conclusion that in this plant the mesocotyl must have a great sensitivity.

The auxin in seedlings of grasses is produced in the tip of the coleoptile; consequently a high auxin concentration is found in the

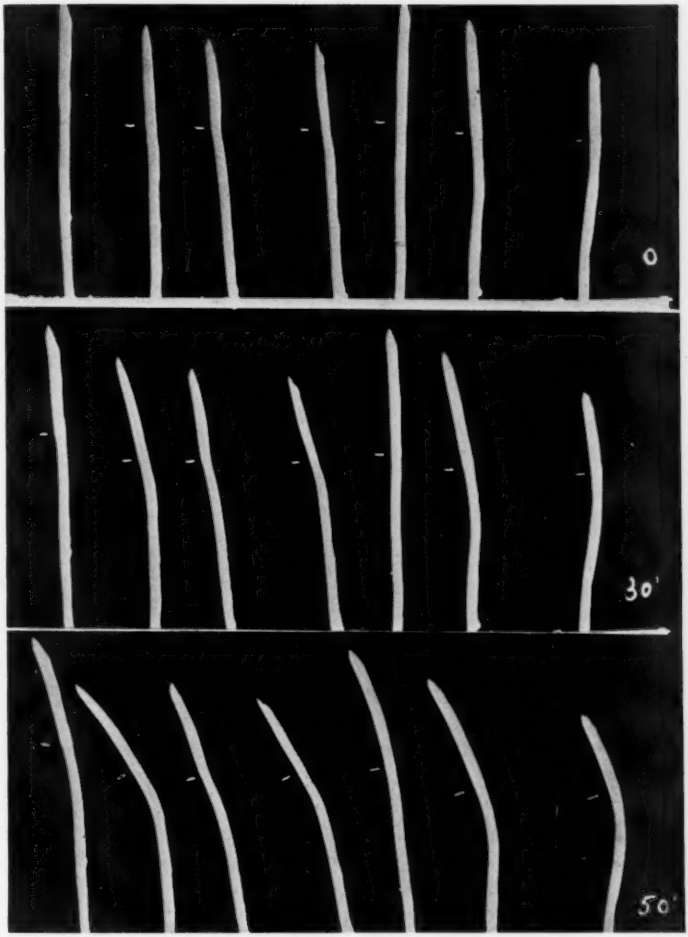


FIG. 2.—Auxin paste (0.0036% indoleacetic acid) smeared along whole right side of 4-days-old corn seedlings. Upper, immediately after auxin application; other rows, 30 and 50 minutes after auxin application. White lines at left side of each plant indicate location of coleoptilar node. No. 61005.

upper part of the coleoptile (figs. 3, 4), decreasing rapidly toward the base. In the upper part of the mesocotyl generally a minimum is found. The coincidence that the minimum for auxin concentration is in the same region as the maximum for growth has already been

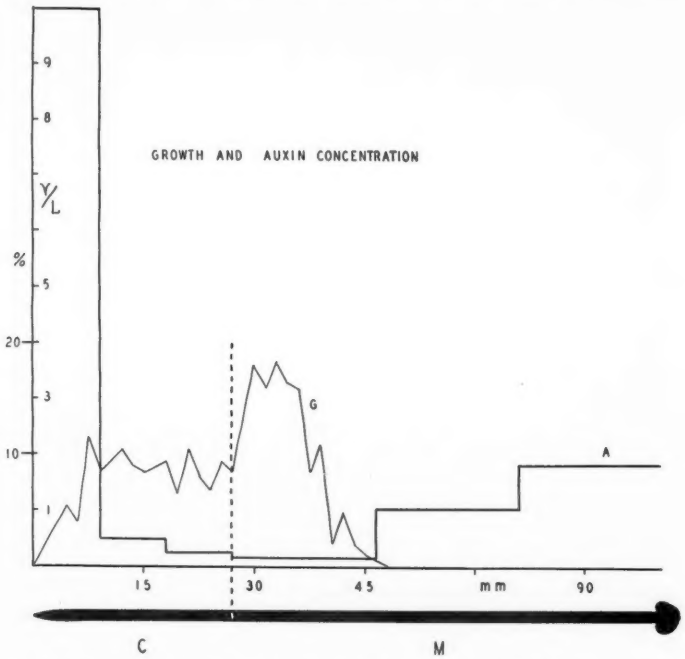


FIG. 3.—Growth and auxin distribution of 5-days-old maize seedlings. Growth in percentage increase in length of 1.5 mm. long segments in 2 hours (curve *G*). Auxin concentration in gammas indoleacetic acid equivalents per liter water contained in the plant (curve *A*). Nos. 70201, 71013, 17022, 71020.

discussed, and was attributed to the great sensitivity to auxin in this region.

From the apical to the basal regions of the mesocotyl the auxin concentration increases. In this basal region, at the time the analyses were made, growth had stopped completely and also there is no response to applied auxin (fig. 2). The theory that the decrease in response to auxin in the basal regions of plants is due to their de-

creased auxin content (**33**, p. 75) does not seem to hold in this case. This relatively high auxin content in the basal regions of the mesocotyl may be linked with the abundant root formation in those regions.

As an explanation of the relatively high auxin concentrations at the basal part of the mesocotyl, the following possibilities may be considered.

1. Auxin produced in the upper parts of the plant may accumulate in the lower portion of the mesocotyl. If this were the case a de-

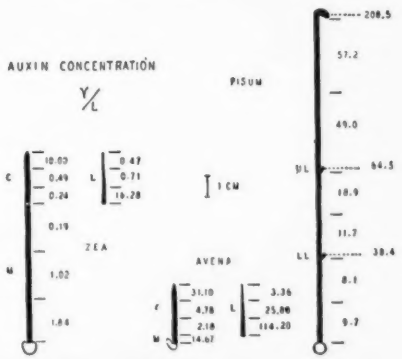


FIG. 4.—Auxin concentrations of etiolated seedlings of corn, oats, and pea, expressed in gammas indoleacetic acid equivalents per liter water contained in the plant. Corn was 5 days, oats 3 days, and peas 7 days old when analyzed, which are stages of development at which these plants are mostly used in the laboratory. *c*, coleoptile; *m*, mesocotyl; *l*, primary leaf; *ul*, upper lateral bud; *ll*, lower lateral bud. Buds in reality are inserted at opposite sides of the stem.

creased auxin production should correspond to a decreased amount of auxin in the basal portion of the coleoptile. Table 3 shows the auxin content of intact plants and plants twenty hours after decapitation, and also of plants from which the roots had been removed. Decapitation consisted of the removal of the upper 1 or 2 mm. of the coleoptile tip; in this way the center of auxin production is removed. Later the auxin production is resumed but to a lesser extent. Removal of the root system of a seedling also leads to reduced auxin production (**22**). With both treatments the auxin content of the coleoptile and the upper region of the mesocotyl is

reduced (table 3). In the base of the mesocotyl, however, the auxin content seems increased. This makes it highly improbable that the relatively high content in the base of the mesocotyl is due to accumulation of auxin produced in the upper regions of the plant.

2. It may be possible that the auxin is carried from the seed into the basal regions of the mesocotyl by the root-pressure stream. The seeds of 5-days-old seedlings carry large amounts of auxin. LAIBACH

TABLE 3

EFFECT OF DECAPITATION AND DEROOTING ON AUXIN DISTRIBUTION OF MAIZE SEEDLINGS. COLEOPTILE TOP OR ROOTS CUT OFF 20 HOURS BEFORE ANALYSIS (NOS. 70903, 6, 8, 9, 13, 16)

PART OF PLANT ANALYZED	AUXIN CONTENT OF 100 PLANTS IN DEGREES OF CURVATURE IN AVENA TEST		
	INTACT	DECAPITATED	DEROOTED
Combined coleoptile and leaf			
Upper half	7.1	4.4	0.4
Lower half	4.6	1.4	0.0
Mesocotyl			
Upper third	5.1	2.1	0.0
Middle third	3.8	3.6	3.0
Lower third	8.9	11.7	11.4
Seed	7.1	.....	.....
Roots	6.0	.....	.....

and MEYER (6) found auxin still present in the kernels of 5-weeks-old corn plants. Although auxin may be present in the seed, the root-pressure stream apparently does not affect the auxin content of the basal regions of the mesocotyl (table 3). However, the possibility that active auxin is transported from the seed to the mesocotyl in some other way still exists.

3. An activation of the auxin precursor into active auxin in the middle and basal regions of the mesocotyl may take place. The precursor was studied by SKOOG (11) in *Avena*. It is transported from the seed to the top of the plant. Such a transport might be independent of the root-pressure stream. This would also explain

why the auxin content of the mesocotyl base seems higher in the derooted and decapitated plants than in the intact ones. In the former two groups of plants the total auxin production is reduced; hence less precursor is used, leaving a higher concentration in the basal regions which respond with an increased production of auxin.

Very little attention has been paid to the auxin content of the primary leaves of the seedlings of *Avena* and maize. At the stage of development in which the plants were analyzed this leaf is still inclosed in the coleoptile. It was thought for a long time that the primary leaves of *Avena* did not contain auxin (**11**). SKOOG was the first to demonstrate auxin in these leaves, by diffusing auxin from the basal part into agar blocks and by testing for auxin with his sensitive deseeded method. The amounts of auxin obtained were so minute that the standard *Avena* test was not sensitive enough to detect them. Thus it was surprising to find that auxin concentrations in the primary leaves of corn and *Avena* seedlings were higher than in any other part of the seedling (fig. 4). The tips of the primary leaves show a relatively low auxin concentration, making the auxin distribution in the primary leaves just the reverse of that in the coleoptiles. The auxin in the leaf must not be readily transportable, which is indicated by SKOOG's experiments and by figures 3 and 4. In these figures it is shown that the minimal auxin concentration is found in the apical region of the mesocotyl. If any appreciable amount of auxin diffused from the primary leaf, the minimum would not be found immediately under its place of attachment.

DISTRIBUTION OF AUXIN IN AVENA SEEDLINGS

THIMANN (**14**) studied the distribution of auxin in *Avena* seedlings, using his acid and chloroform method. With the ether extraction method and without acidifying, results were obtained which closely correspond to his. Figure 4 shows that in the upper region of the coleoptile a concentration was found corresponding to 31.10 gammas indoleacetic acid per liter water contained in the plant material. From THIMANN's figures (**14**, p. 32, and **33**, p. 68) and from his table V (**33**, p. 43), it was calculated that the auxin concentration of the upper third of the coleoptile corresponds to values

between 9.4 and 22.2 gammas indoleacetic acid per liter. These values compare favorably with the one (31.10) shown in figure 4. An accurate comparison was impossible because the sensitivity of THIMANN's test plants is unknown. The values shown in figure 4 should not give the impression that the auxin concentrations given there are constants. In the first place, the auxin concentration varies with the stage of development (see for instance figure 5). Then, even though the plants were grown in physiological darkrooms under

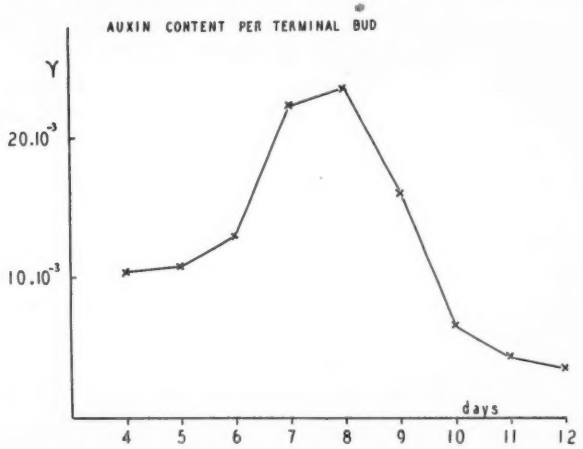


FIG. 5.—Auxin content in indoleacetic acid equivalents of terminal pea buds as a function of age of etiolated seedling. At 4 days the buds have just emerged above surface of the sand in which they are planted. Nos. 70920, 70922, 71005, 71006.

constant temperature and humidity, there still was considerable variation in auxin content from day to day. It was found that these uncontrollable fluctuations were much more pronounced in pea plants than in *Avena* seedlings.

For the lower part of the *Avena* seedlings, THIMANN found values which are higher than the ones shown in figure 4. Calculated from his data, values between 3.6 and 8.6 gammas indoleacetic acid equivalents were found. This may be due to his way of analysis. From his illustrations (**14,** fig. 2 and **33,** fig. 30) one can conclude that the mesocotyl was included in the analysis of the basal regions. Figure 4 shows clearly that the mesocotyl has a much higher auxin

content than the basal part of the coleoptile. Mesocotyls of plants grown in glass holders with their roots in water are usually not longer than 3 to 4 mm. The mesocotyl length depends very much upon the amount of light it gets (**1, 20**). If grown in complete darkness, mesocotyls of several inches long may develop. If grown in sand with the seeds sufficiently covered, mesocotyls develop which are longer than the ones grown in water cultures. Table 1 gives the analyses of two sets of plants, one grown in glass holders and the other in sand. The upper parts (coleoptile and primary leaf) of the plants of both sets are identical as far as their auxin content is concerned. The mesocotyl of the sand-grown plants was 24 mm. long and showed an auxin distribution very similar to that of corn mesocotyls. At the apical regions it is low and in the basal regions of the mesocotyl it is high. No special experiments have been carried out to determine whether the high auxin content of the primary leaf in some way affects the auxin concentration of the mesocotyl. From the distribution of auxin in the mesocotyl of sand-grown plants, however, it appears that this is not the case, and that the high auxin content of the mesocotyl base should be attributed to auxin or its precursor coming from below.

Part II: Auxin concentrations and bud inhibition in Pisum

The pea variety Alaska was used throughout these experiments. The seeds were soaked for a few hours in water and planted in moist sand in a physiological darkroom at 24° C. and 90 per cent humidity. At the age of about seven days (from the time of soaking) the plants were ready to be used. The intact pea plants (etiolated) at that time have an auxin content as shown in figure 4. The terminal bud has an auxin concentration which is much higher than the concentrations found in the tips of corn or oat coleoptiles. This was surprising because by the diffusion method much more auxin can generally be obtained from corn and oat tips than from terminal buds of pea seedlings. WENT and THIMANN (**33**, p. 82) state that only small amounts of auxin have been obtained from apical buds of etiolated pea plants by the diffusion method. As figure 4 shows, the terminal bud has the highest auxin concentration of the etiolated pea seedling. Toward the base the concentration decreases. The dormant

lateral buds have a higher concentration than the adjacent stem tissue. The buds have been extracted without any part of the stem attached to them. The stipules, for technical reasons, were analyzed with the buds.

The auxin content of the seedlings varies with their age. Figure 5 shows the content per terminal bud at stages of development between four and twelve days after the seeds were soaked.

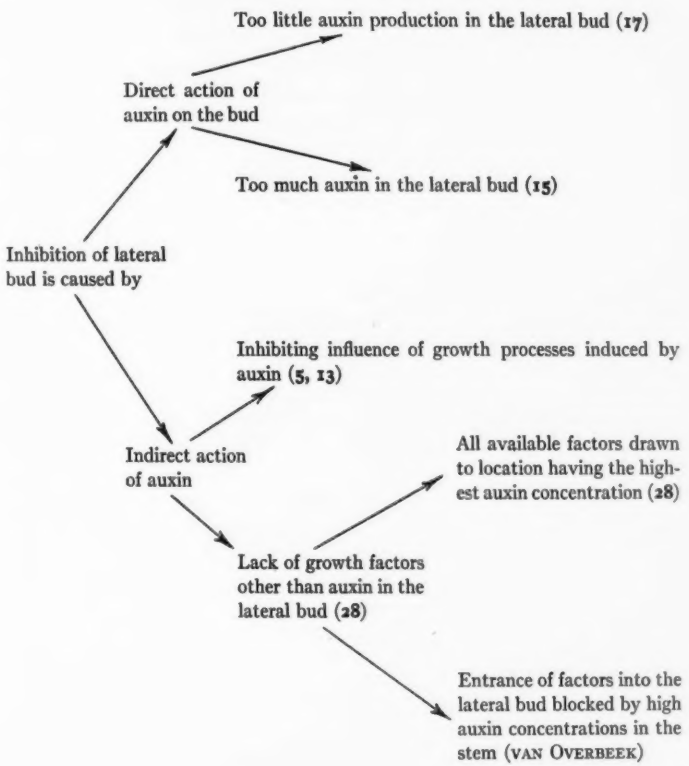
After the terminal bud of a pea seedling is removed, a lateral one starts to develop. In the pea this lateral bud generally is the upper one (figs. 4, 6). This inhibitory influence of the terminal bud upon the lateral ones has been known for a long time; it forms the basis of pruning. THIMANN and SKOOG (**16**) were the first to demonstrate that this inhibitory influence of the terminal bud is nothing but the auxin produced by it. A little later LAIBACH (**5**) demonstrated the same. THIMANN and SKOOG removed the terminal bud and put a dosage of auxin on the stump. The buds did not start to grow, but if the auxin was removed the lateral bud developed. This would indicate that auxin is able to prevent lateral buds from developing.

To explain this effect on bud inhibition, several theories have been advanced. Some of them try to explain it on the basis of a direct action of auxin on the lateral bud, others consider the effect of auxin as indirect. The scheme on the opposite page may be used to illustrate these various theories.

There are two groups of theories, one favoring direct action of auxin on the lateral bud and another favoring indirect action. To the first group belong the theories of THIMANN and SKOOG and a recent one of THIMANN. According to the first theory (**17**), the auxin produced by the terminal bud reaches the lateral buds and prevents their own production of auxin. As soon as the terminal bud is removed the lateral buds commence to synthesize auxin on their own account, which makes the buds develop. The authors of this theory showed that from dormant buds of *Vicia* no auxin could be obtained by the diffusion method. If the lateral buds started to develop large amounts of auxin could be found.

The recent theory of THIMANN (**15**) postulates that the relation between auxin concentration and growth can be expressed as an

optimum curve. Roots, buds, and stems all have their optimum at different concentrations. According to this theory the buds are inhibited at a concentration of $10^{-6.7}$ molal or higher. The bud growth is stimulated, according to THIMANN's scheme on page 411,


Too little auxin production in the lateral bud (17)
Direct action of auxin on the bud
Too much auxin in the lateral bud (15)
Inhibition of lateral bud is caused by
Inhibiting influence of growth processes induced by auxin (5, 13)
Indirect action of auxin
All available factors drawn to location having the highest auxin concentration (28)
Lack of growth factors other than auxin in the lateral bud (28)
Entrance of factors into the lateral bud blocked by high auxin concentrations in the stem (VAN OVERBEEK)

at a concentration lower than $10^{-6.7}$ molal (which corresponds to 37 gammas indoleacetic acid per liter). In other words, when a lateral bud will grow out it must have a lower auxin content than when it stays dormant, according to this theory (15). This auxin content of the lateral bud at different stages of development can easily be determined with the extraction method, and in this way the validity

of the theory (15) can be tested. This is done below; figures 6, 7, and 8 give the results. They show that after decapitation the auxin content and concentration of the lateral buds increases over that in the dormant stage. As soon as twelve hours after decapitation the first increase in auxin content was observed. Figure 6 shows that during

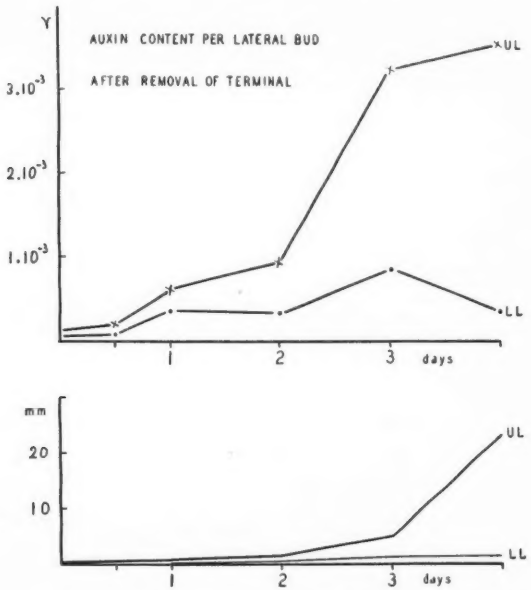


FIG. 6.—Auxin content (indoleacetic acid equivalents) and length of lateral pea buds after removal of terminal bud. Abscissa: time after decapitation of terminal bud. Nos. 70915, 70917, 70920, 70921, 70922, 70923, 70924, 71002, 71007, 71008a.

the first twenty-four hours after decapitation, both the upper and the lower lateral bud increased in auxin concentration. They also increased in weight (table 4). From that time on the upper bud steadily increases its auxin content, while the lower one (the one that does not develop) fails to show further increase. Four days after decapitation the upper lateral bud has an auxin content corresponding to between 3 and 4 $\times$ 10^{-3} gamma indoleacetic acid. This is the amount the terminal bud would have had if the plant had not been

decapitated (11-days-old plants of figure 5). It may be observed that the great increase in growth of the upper lateral bud (fig. 6) is preceded by a sharp increase in auxin content in this bud.

Figure 7 shows the auxin concentrations in pea plants twenty-four hours after decapitation. Three sets with about 150 uniform pea seedlings in each set were selected. One was kept intact (*N*), another was decapitated about 2 cm. above the upper lateral bud (*D*), a third was also decapitated but followed by application of 0.1 per cent indoleacetic acid in lanolin to the cut stump (*DA*). Of each set, seventy-five plants were analyzed for auxin concentrations while the remaining ones were used for weight and inhibition determinations. The paste containing 0.1 per cent indoleacetic acid proved to give a complete inhibition of the laterals. Figure 7 shows the average result of two identical experiments. The same data are used in figure 8 where the relative changes in auxin due to decapitation and decapitation followed by auxin application are shown (intact plants 100 per cent). The effect of decapitation is seen by comparing the *N* and *D* curves and the diagrams of figures 7 and 8. In the stem the auxin concentration decreases after decapitation; in the lateral buds it increases.

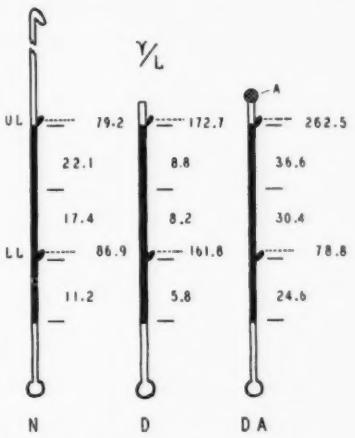


FIG. 7.—Auxin concentration (gammas indoleacetic acid equivalents per liter water contained in the plant) of pea seedlings 24 hours after decapitation and auxin application at cut surface of stump (*DA*), of seedlings that were left intact (*N*), and of seedlings decapitated without additional auxin application. Auxin applied: 1 gamma indoleacetic acid per 1000 gm. of lanolin. Of all the lateral buds shown, upper lateral one (*UL*) of *D* is only one that will develop. Nos. 71007, 71008a, 71014.

This is in agreement with the results obtained by THIMANN and SKOOG (17) with *Vicia*. They found that in the undeveloped lateral buds the auxin production was only 1.6 plant units per hour; in the developing laterals it was found to be 19.2. It is this increase in

auxin production which according to the theory (17) is responsible for the growing out of the lateral buds. From figures 6 and 7 it follows that this increase in auxin production increases the auxin content and the auxin concentration of the lateral buds. Still, any increase in auxin concentration in the lateral buds to a concentration range found in the developing buds does not make the lateral buds grow out. This should be the case if the theory were valid. In fig-

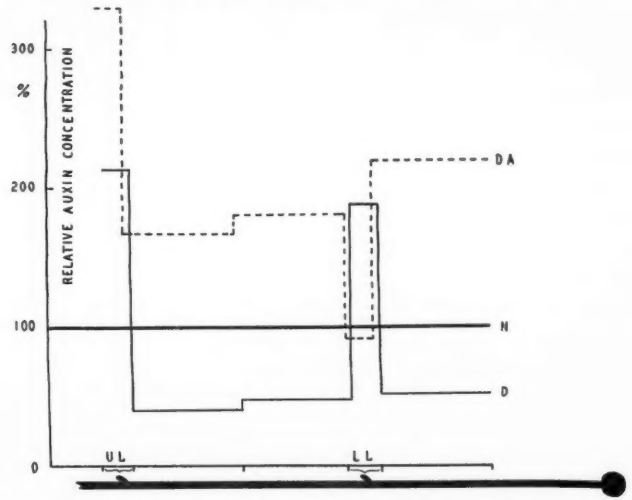


FIG. 8.—Effect of decapitation (*D*) and decapitation followed by auxin application (*DA*) on auxin concentration throughout etiolated pea plants. Auxin distribution shown was found 24 hours after decapitation. Intact plants 100%. Nos. 71007, 71008a.

ures 7 and 8 auxin concentrations are given of plants which were decapitated and on the stump of which auxin dissolved in lanolin was applied (*DA*). The auxin concentration in the stem of these *DA* plants is increased over that in the intact and decapitated plants. The auxin concentration of the upper lateral bud of the *DA* plants is higher than that in the intact plants, and even higher than in the upper lateral bud of the decapitated plants. Nevertheless this upper lateral bud (*DA*) fails to grow out, from which it may be concluded that a mere lack of auxin production, hence lack of auxin, is not the cause of the failure of the dormant lateral buds to develop. This

conclusion is not valid if one assumes that the lateral buds produce a kind of auxin which has a physiological activity completely different from indoleacetic acid. This is extremely unlikely, since one would expect an auxin of the type of auxin-a to be produced in pea plants. The conclusion seems evident that bud inhibition cannot be due to a direct action of auxin on the bud. Hence, bud inhibition can be due neither to relatively high auxin concentrations in the lateral bud

TABLE 4

WEIGHT OF SEVENTY-FIVE LATERAL BUDS 24 HOURS AFTER REMOVAL OF TERMINAL BUD

	Weight in milligrams	
	Intact	Decapitated
Upper lateral bud		
Fresh	193.7	283.0
Dry	23.1	35.1
Lower lateral bud		
Fresh	139.3	181.6
Dry	15.4	22.4

nor to relatively low auxin concentrations or low auxin production in these buds.

In figures 7 and 8 it is shown that the effect of decapitation on the distribution of auxin is twofold. The auxin concentration in the lateral buds increases, while in the stem it decreases. Since the bud inhibition of the type discussed here can undoubtedly be controlled by auxin, and since the conclusion was reached that the auxin concentration in the lateral buds themselves does not directly determine whether such bud will develop or not, it follows that a relatively low auxin concentration in the stem may be the important factor responsible for the development of the lateral bud, and a relatively high auxin concentration in the stem apparently prevents the lateral buds from developing. This conclusion is also supported by the following experiments by Skoog (10). He reduced the auxin content of the plants by irradiating with x-rays, and found that the more auxin destroyed (within certain limits) by irradiation, the bet-

ter was the development of the lateral bud. He excluded the possibility of stimulation of the laterals by the x-rays by shielding the buds.

How a high concentration of auxin in the stem may prevent the development of the lateral buds is the next question to be considered. The following experiments may lead to its explanation. A ring of lanolin paste in which 0.1 per cent indoleacetic acid had been dissolved was applied around the stem below the lateral bud of decapitated pea plants. The lateral bud developed. If a ring of the paste was applied 2 cm. above the upper lateral bud this bud also grew out. If, however, the paste was applied at the apical cut surface 2 cm. above the upper lateral bud, this bud was completely inhibited. The conclusion may be drawn that in order to be effective the auxin (of the concentration employed) must be introduced in the vascular system of the stele. The epidermis, cortical tissue, and endodermis apparently impede the auxin transport in a lateral direction to a considerable degree. It is clear that auxin of a concentration higher than the one employed here may very well be able to inhibit lateral buds when applied in a ring around the stem above these buds.

Another experiment to be considered is the one already shown in figure 7. If the auxin concentration of the lower lateral bud of the *DA* set is compared with that of the same bud of the *N* set, it is seen that these concentrations are practically the same. Repetition of the experiment brought the same results. Apparently a relatively high auxin concentration in the stem prevents the auxin formation in the lateral bud. To study this, one must be able to distinguish between the auxin produced by the bud and the auxin introduced by application. This was found possible by using phenylbutyric acid instead of indoleacetic acid. WENT found that this substance has not the slightest activity in the *Avena* test, yet it is able to inhibit bud development to a considerable degree (table 5). WENT (30) found that there are several substances which have only part of the properties found in auxins. With this phenylbutyric acid an experiment similar to the one of figure 7 was made. Its result is recorded in table 6. The phenylbutyric acid had to be applied in rather high concentrations in order to be effective. Furthermore, it does not penetrate

into the plant as readily as does indoleacetic acid. Applied at a distance of 2 cm. above the upper lateral bud it is hardly effective. Applied at about 1 cm. above the upper lateral bud it inhibits development of this bud to a marked degree. Table 6 shows that the auxin concentration in the upper lateral bud of the decapitated plants to which phenylbutyric acid had been applied does not increase. Hence the formation of auxin in the lateral bud is prevented

TABLE 5

LENGTH IN MM. OF LATERAL BUDS OF PLANTS OF TABLE 6 MEASURED FOUR DAYS AFTER DECAPITATION
AVERAGE OF 30 PLANTS EACH

LATERAL BUD	*N*	*D*	*DP*
Upper	0	18.5	6.7
Lower	0	0.7	6.9
	PERCENTAGE HAVING LENGTH BELOW 2 MM.		
Upper	100	0	38
Lower	100	86	20

TABLE 6

AUXIN CONCENTRATIONS OF THREE SETS OF ETIOLATED PEA SEEDLINGS. *N*, INTACT; *D*, DECAPITATED; *DP*, DECAPITATED FOLLOWED BY APPLICATION OF 2% PHENYLBUTYRIC ACID PASTE. ANALYSES MADE 24 HOURS AFTER DECAPITATION. CUT SURFACE AND PASTE APPLICATION 1 CM. ABOVE UPPER LATERAL BUD. CONCENTRATIONS IN GAMMAS INDOLEACETIC ACID EQUIVALENTS PER LITER (NO. 71208)

	N	*D*	*DP*
Upper lateral bud	104.3	277.0	94.0
Upper part of internode between buds	9.4	4.2	0.98
Lower part of same internode	1.37	1.8	0.70
Lower lateral bud	0.0	0.0	156.3

by a high concentration of this acid. This confirms the preliminary conclusions drawn from the low auxin concentration of the lower lateral bud of figure 7 *DA*, that a relatively high concentration of auxin in the stem prevents the formation of auxin in the lateral buds.[1]

The lower lateral buds of the plants treated with phenylbutyric acid (table 6 *DP*) show a high auxin content compared with the lower buds of the sets *N* and *D*, which was abnormally low in this series of plants. In the table the auxin content of these buds is given as 0.0; this does not necessarily mean that the buds were devoid of auxin, but that for the number of buds extracted (seventy-five) and for the volume of agar in which the dry extract was taken up (0.5 cc.) no curvature could be obtained in the *Avena* test. It may well be possible that if the number of buds extracted had been larger a small curvature would have been obtained. It was mentioned in the first part of this paper that even though the seedlings were grown under controlled temperature and humidity conditions, still considerable fluctuations in growth and auxin content could be observed from day to day.

The relatively high auxin content of the lower lateral bud of the *DP* set may be accounted for as follows. If less precursor is used for the auxin production of the upper lateral bud (*DP*) than for the more rapidly developing upper lateral bud of the set *D* (in the set *N* the precursor is used for auxin production in the terminal bud), more is left over for the auxin production of the lower lateral bud of the plants of the set *DP*. The lower lateral bud is able to develop because, as has been shown, the phenylbutyric acid penetrates with difficulty into the stem and is not present in high enough concentrations in the lower part of the plants to prevent bud development.

If one assumes that the auxin production in the lateral buds does not take place because auxin precursor is lacking, the problem is reduced to the following considerations: Relatively high auxin concentrations in the stem prevent the auxin precursor from entering the lateral buds. If the auxin precursor cannot enter the bud it is also likely that other factors necessary for growth of the buds are not

[1] FERMAN (Rec. Trav. Bot. Néerl. **35**: 177–287. 1938) came to the same conclusion in a different way. Similar conclusions may be drawn from SKOOG's (Amer. Jour. Bot. **25**: 361–372. 1938) table 5.

able to enter. WENT has assumed that bud inhibition is due to lack of specific growth factors (see scheme on page 149). He believes these factors to be hormones. For the present consideration the nature of those growth factors is of secondary importance. With grafting experiments, WENT (**29**) showed that the factors move up through living tissue of the vascular system. The vascular system of the pea at the stages of development as used in the present experiments has been studied by GOURLEY (**3**). He found that in the stele a number of large vascular elements are present. The dormant lateral bud is connected by means of only a small trace to this stelar system. Cortical bundles have no connection with the dormant lateral bud. MORELAND (**8**) showed that in bean plants the vascular supply leading to the cotyledonary buds is normally very weakly developed. He also showed that within forty-eight hours after decapitation this vascular supply has been increased.

It has already been shown that a high concentration of auxin in the stem prevents the lateral buds from developing, and that in order to be effective the auxin has to be introduced to the vascular system. So one might conclude that a relatively high auxin concentration in the vascular system of the stem is necessary for inhibition of the lateral bud.

One way to visualize the action of auxin in the vascular system is the following. Auxins are surface active substances. Hence, by entering the vascular system their molecules may get adsorbed to the walls of the vascular elements (phloem or xylem or both).[2] Suppose the materials necessary for growth are translocated from the seed to other parts of the plant through the vascular system by means of a mass flow. The velocity of such a flow will be affected by the properties of the wall of the vascular element and by the diameter, as the flow of water through a capillary tube is affected by the properties of the wall and the diameter. If the auxin is adsorbed to the wall of vascular elements, no doubt the physico-chemical properties of the wall will be changed; hence the mass flow through such a vascular element will be changed.

That auxin has an effect on the flow of protoplasm in cells is beyond doubt. This has been shown by THIMANN and SWEENEY

[2] The phloem is likely to be the most important in this consideration.

(18), who showed that in low concentrations auxins promote the velocity of protoplasmic streaming; in high concentrations the streaming is slowed down. If one assumes that the flow of food materials through the phloem elements is slowed down likewise by high auxin concentrations, then it will be clear that the effect of auxin (adsorbed on the wall) will be greater in small narrow elements than in wide ones. In other words, the flow of food material will be only slightly interfered with in the main bundles of the stele but through the narrow trace to the dormant lateral bud it will be impossible. Thus growth factors may be prevented from reaching the dormant bud, but allowed to pass through the large bundles to the growing region of the stem.

The validity of this conclusion can be tested in the following way. If the laterals are allowed to develop, the vascular system develops with them. MORELAND (8) showed that decapitation of the terminal bud or any other treatment leading to development of the lateral buds is followed by increased vascular supply to the lateral buds. He showed that prior to expansion of the buds their vascular connections have been strengthened. The effect of auxin on lateral buds with such an increased vascular supply, on the basis of the preceding considerations, can be expected to be less the more the lateral bud has developed. This indeed is what happens. It was known to THIMANN and SKOOG that after the laterals had grown out to a certain length, auxin when applied to the main stem was without effect on these laterals. Figure 9 shows how the growth of the upper lateral buds is influenced by auxin applications (in the way of figure 7 *DA*), made when the laterals had reached different stages of development. Curve *D* is the growth curve of laterals of decapitated plants to which no auxin had been applied. Curves 0, 1, 2, and 3 are growth curves of decapitated plants to which 1 per cent indoleacetic acid in lanolin was applied after the laterals had been allowed to grow for respectively 0, 1, 2, and 3 days. It is clear that the effect of applications of auxin to the stump of the stem is less the more the growth of the lateral has advanced. This not only is elongation of the lateral bud, because two days after decapitation, when the auxin is hardly able to influence the growth, the lateral bud has barely increased in length (fig. 6). What is important here is that prior to

expansion of the lateral bud its vascular supply has been increased, as found by MORELAND.

The experiment of figure 9 was also done with auxin pastes containing 0.1 and 0.02 per cent indoleacetic acid. The results were the same, although of course the inhibition is much less with weaker

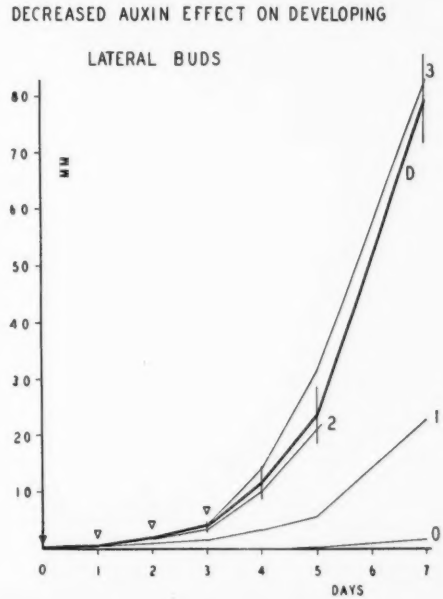


FIG. 9.—Length of upper lateral bud in relation to time after decapitation. Indoleacetic acid applied to cut surface of stump after buds had been allowed to develop for 0, 1, 2, 3, days (curves 0, 1, 2, 3). Heavy curve *D* is of plants to which no auxin was applied. Indoleacetic acid paste 1%. Inverted triangles indicate time of auxin application in the various sets. Thin vertical lines indicate range of variation for curve *D*. Averages of about 30 plants. No. 71220.

pastes than with stronger ones. The plants of curve 2 were taken out of the experiment after the fifth day (abscissa) and analyzed for their auxin concentrations. The results are shown in figure 10. In the decapitated plants without auxin paste the highest auxin concentration is found in the top of the developing lateral. In the other cases, especially in the one where 1 per cent indoleacetic acid was

applied, the highest auxin concentration is found in the upper part of the main stem. If a situation like this exists, then according to WENT's theory (see scheme on page 149) all the growth factors limiting the growth of a lateral bud move to the site of the highest auxin concentration, which is the upper part of the main stem. Hence, according to this theory the laterals of the set *DA* 1 of figure 10 should be inhibited. This does not happen.

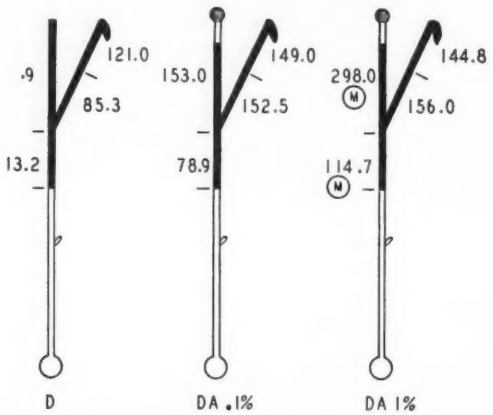


FIG. 10.—Auxin distribution (gammas indoleacetic acid equivalents per liter water contained in the plant) of pea plants to which auxin had been applied after laterals had been allowed to develop for 2 days. Analyzed 3 days after auxin application. In set *D* no auxin was applied. In *DA* 0.1, 0.1% indoleacetic acid was applied and in set *DA* 1, 1% was applied. The *DA* 1 set was analyzed where curve 2 of figure 9 is terminated. The *M* in circle next to a figure means that given value is too low because of a maximum angle in the assay. No. 71221.

SNOW (13) assumes that the formation of an inhibitory substance caused by the action of auxin is a growth process. He investigated the lateral buds of *Vicia* and pea plants grown in light, and believes that the inhibiting substance is able to spread throughout the plant regardless of polarity. He based this view on the assumptions that auxin is unable to move in the plant in a morphologically upward direction, and that the base of the inhibited shoot of his plants is free of auxin. Figure 10 shows that the auxin applied at the top of the main stem may be able to move upward in the developing lateral.

Indeed LE FANU (7) has not been able to obtain auxin by diffusion from inhibited pea shoots. This, however, does not prove that the shoot does not contain auxin. The experiments with primary leaves of *Avena*, mentioned in the first part of this paper, have shown that large amounts of auxin are present in these leaves although only extremely small amounts can be obtained from them by diffusion.

In the pea seedling as a rule the upper lateral bud develops after decapitation while the lower remains dormant (fig. 6). This may be explained by the following considerations. Table 7 shows the results of an experiment in which the stem of the pea seedling was either cut

TABLE 7

PERCENTAGE OF LATERAL BUDS THAT HAVE STARTED TO DEVELOP WITHIN TWO DAYS AFTER MAIN STEM HAD BEEN CUT OFF 2 CM. ABOVE EITHER UPPER OR LOWER LATERAL. AVERAGES OF ABOUT 40 PLANTS

DECAPITATION	NUMBER	PERCENTAGE CONCENTRATION OF INDOLEACETIC ACID PASTE				
		0	0.005	0.01	0.05	0.1
Above upper lateral bud (upper buds).	71222	74	81	67	5	0
	71228	81	71	22	0	.....
Above lower lateral bud.....	71222	21	13	5	0	0
	71228	20	14	0	0	.....

off 2 cm. above the upper lateral bud or 2 cm. above the lower one. If no paste containing auxin was applied to the stump, of the upper buds about 75 per cent had started to grow out within the first two days after decapitation, while only 20 per cent of the lower buds were developed in the plants which were decapitated 2 cm. above this bud. So the upper bud is about two days ahead in development. From figure 6 it may be seen that this is exactly the stage at which the upper bud starts to produce large amounts of auxin, which flows into the main stem (fig. 10*D*) where it raises the concentration to a point where it inhibits growth of the lower lateral bud. There is also evidence that low auxin concentrations are more effective in inhibiting bud development of the lower lateral bud than of the upper. This evidence is presented in table 8. Here two experiments are given. In

experiment 71222 an auxin application of 0.01 per cent indoleacetic acid in lanolin does not inhibit development of the upper lateral bud, while the length of the lower one is reduced to 50 per cent of the length of the controls. In experiment 71228 a similar effect is seen at a still lower concentration.

In conclusion, it may be helpful to summarize the facts available for the explanation of bud inhibition. After decapitation, two changes take place in the auxin content of a seedling: (1) the auxin production (**17**) and concentration (figs. 7, 8) of the lateral bud increases; (2) the auxin concentration in the stem decreases (figs. 7, 8). The increase in auxin content of the lateral buds, however, is not the direct reason for the development of these buds (fig. 7*DA*), thus ruling out a direct action of auxin on the lateral bud as the cause of bud inhibition, and making it likely that the controlling action is on the stem rather than on the lateral bud itself. A reduction of the auxin content of the stem by other means than decapitation also was shown (**10**) to result in development of the lateral buds. It was shown that auxin must be introduced into the vascular system of the stele in order to be most effective. This indicates that the place of action from which the auxin controls development of the lateral

TABLE 8

RELATIVE LENGTH OF UPPER LATERAL BUD AFTER REMOVAL OF STEM UP TO 2 CM. ABOVE THIS BUD (*U*), AND OF LOWER LATERAL BUD AFTER DECAPITATION 2 CM. ABOVE (*L*). INDOLEACETIC ACID APPLIED IMMEDIATELY AFTER DECAPITATION ON CUT SURFACE OF STUMP

EXPERIMENTAL NO.	TIME AFTER DECAPITATION IN DAYS; AND *U* OR *L* SET	PERCENTAGE CONCENTRATION OF INDOLEACETIC ACID PASTE				
		0	0.005	0.01	0.05	0.1
71222	3 (*U*)	100	108	**95**	28	0
	3 (*L*)	100	104	**54**	20	0
	4 (*U*)	100	124	**112**	28	0
	4 (*L*)	100	100	**54**	20	0
71228	3 (*U*)	100	**107**	50	27	.....
	3 (*L*)	100	**65**	60	30	
	4 (*U*)	100	**107**	55	24	.....
	4 (*L*)	100	**80**	65	30	.....

buds must be this vascular system. From the anatomy of the pea seedling it is known (3) that the dormant lateral bud is connected with the main bundles of the stele only by means of a small trace. It was shown (table 6) that high auxin concentrations in the vascular system prevent auxin formation in the lateral buds. It was assumed that this auxin formation did not take place because its precursor was lacking, and that the precursor could not pass through the narrow trace. Since this lack of precursor or auxin was shown not to be the cause of the bud inhibition, it was assumed that the flow of materials necessary for growth (including precursor) is stopped by high auxin concentrations. Such a flow could take place in the phloem. It is known (18) that the flow of protoplasm (protoplasmic streaming) is inhibited by high concentrations of auxin; a similar effect may take place in the vascular bundles on the mass flow. If one assumes that the auxin is adsorbed to the walls of the vascular elements, and from there by means of changing the physico-chemical properties of this wall exerts an effect on the flow of growth materials, it is clear why auxin would be able completely to inhibit translocation through narrow vascular elements and hardly at all in larger wider ones. Thus lateral buds could be inhibited while at the same time growth in the main stem could take place. Support of this view was obtained from the following facts. It was known (8) that any treatment leading to development of the lateral buds is followed by an increase in the vascular supply to these buds. This takes place before the bud grows out and within forty-eight hours after the treatment. Auxin was applied to the stump of plants with lateral buds having such an increased vascular supply and was found to be unable to inhibit the development of these buds (fig. 9).[3]

Summary

1. A method is described by which auxin was extracted from plant materials without the usual addition of acid. Acid was found to

[3] After this paper had gone to press, two articles by FERMAN (FERMAN, J. H. G., Proc. Kon. Akad. Wetensch. Amsterdam **41**:167–180. 1938; Rec. Trav. Bot. Néerl. **35**:177–287. 1938) on the bud inhibition in *Lupinus* were received. This author offers two different explanations, one for the inhibition of intact plants and another for the inhibition with artificial auxin. In intact plants the auxin precursor "is transported acropetally and chiefly attracted to those spots where auxin is most intensely pro-

reduce the yield in some cases (corn) and in other cases (oats) to be of no benefit. The distribution of auxin concentrations in etiolated seedlings of corn, oats, and peas was determined (fig. 4). The amounts of auxin were not expressed in the usual way in arbitrary units, but in indoleacetic acid equivalents which are independent of the sensitivity of the test plant, of the test plant itself, and of the conditions under which the test is made.

2. In corn the distribution of growth was compared with the distribution of auxin (fig. 3). It was found that the region having the greatest growth rate (the upper part of the mesocotyl) has the lowest auxin concentration. This was found to be due to the great sensitivity to auxin in this region.

3. The highest auxin concentrations in the seedlings of corn and oats are found in the basal regions of the primary leaves. This may not be free moving auxin. The average auxin concentration is lowest in corn seedlings and highest in pea plants.

4. The problem of bud inhibition was analyzed in pea seedlings. The effect of decapitation on auxin concentrations in the plant was determined. It was shown that as soon as twelve hours after decapitation the auxin concentration in the lateral buds is increased (figs. 6–8). In the stem the concentration decreases after decapitation. Auxin application increases the concentration in the stem and also in the upper lateral bud. From these analyses it was concluded that a direct action of auxin on the lateral bud is excluded as a possible explanation for its dormancy.

5. From experiments with phenylbutyric acid it was concluded that high concentrations of auxin (or phenylbutyric acid) in the stem prevent the formation of auxin in the lateral bud (table 6).

6. Auxin is most effective in inhibiting lateral buds when introduced into the vascular system of the stele.

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duced." The young axillary buds "remain deprived from the precursor and therefore dormant, since they cannot produce auxin and consequently cannot grow out." For the bud inhibition caused by artificial auxin, FERMAN assumes that indoleacetic acid "prevents or seriously hampers already in the basal parts of the stem the upward movement of the precursor in its tracks of transport." Since both explanations ultimately are based on the thesis that it is the lack of auxin which prevents the lateral buds from developing, they can be criticized in the same way as THIMANN and SKOOG'S (17) theory.

7. Auxin applied to the stump of decapitated plants some time after decapitation is less effective in bringing about bud inhibition the longer the time between decapitation and application (fig. 9).

8. The preceding evidence, in combination with many valuable data obtained from the literature, has provided material with which an attempt was made to explain the auxin controlled bud inhibition in agreement with all known data. A brief explanation as to how the mechanism of this bud inhibition might work, together with the main facts upon which it was based, is given at the end of part II.

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ANATOMY OF AUXIN TREATED ETIOLATED SEEDLINGS OF PISUM SATIVUM

FLORA MURRAY SCOTT

(WITH TWENTY-NINE FIGURES)

Introduction

The anatomy of auxin induced swellings in *Phaseolus* has been described in detail by KRAUS (4) and his collaborators, and BORTHWICK (1) and his colleagues have given a full account of similar tumors in *Solanum*. The present investigation is concerned with the reactions of the stem of the etiolated seedling of *Pisum sativum* on treatment with a known auxin, indole(3)acetic acid.

Material and methods

Three series of experimental plants were grown: series A and series B were treated with auxin paste, while series C was treated with aqueous solutions of auxin. The seedlings were grown and treated under standard experimental conditions in the laboratory.

SERIES A.—Seeds of the garden pea, *Pisum sativum* (variety Alaska), germinated and grown in the dark under constant conditions of temperature and humidity (24° C. and 85 per cent humidity), developed into typical etiolated plants of the type used by WENT (7) in the standard pea test. When the first foliage leaves began to unfold at the fourth internode, the seedlings were decapitated 1 cm. below this node and the cut surfaces smeared thickly with lanolin-auxin paste (1 gm. indole(3)acetic acid in 100 gm. lanolin). Blunt conical swellings developed in the course of a few days, in the tissues of which root primordia arose (fig. 1).

Control seedlings were grown under exactly similar conditions of darkness and temperature. Some were decapitated and left untreated, others decapitated and treated with lanolin, while a certain number were allowed to continue normal development. No control seedling stem showed any trace of swelling beneath the cut surface. In the treated seedlings, on the other hand, increase in diameter was apparent within the first twelve hours. In seventy-two hours a club-

shaped swelling extended about 8 mm. down the stem, and continued to enlarge during the next few days. The vascular tissues of the control seedlings, decapitated and non-decapitated, developed in the normal way, but differed markedly in extent. A comparison of typical transverse sections of decapitated control and auxin treated internodes indicates this contrast clearly (figs. 23, 24).[1] Collections of seedlings, both treated and control, were made at eight hour intervals up to ninety-two hours, and thereafter less frequently up to 164 hours. The material was infiltrated with Flemming's or with Navashin's solution, imbedded, sectioned, and stained with safranin and fast green or with haematoxylin. Hand sections, treated with iodine potassium iodide, with thionin, or with phloroglucin and hydrochloric acid, were used in the examination of the starch content and the lignification of the endodermis and other tissues. The sugar content was also tested by means of Fehling's solution. Since sugar was abundant throughout the duration of the experiment, this test was of no value from a quantitative standpoint.

SERIES B.—A second series of seedlings was grown to test the effect of age on the swelling of the internode. The seedlings were grown under constant conditions as before, but were not decapitated and treated until they were a week older. Swelling and root formation were slight or even entirely lacking in many specimens (fig. 2).

SERIES C.—Auxin in aqueous solution was used in this series, for a comparison of auxin-water and auxin-paste induced swellings. Various concentrations of auxin were used: (1) 100 mg. per liter; (2) 200 mg. per liter; (3) 400 mg. per liter; (4) 1000 mg. per liter. The resultant swelling and root formation varied markedly according to concentration (figs. 3, 4).

Observations

ANATOMY OF THIRD INTERNODE

The anatomy of the seedling pea, Champion of England, has been described in detail by GOURLEY (2). The root-stem transition region extends through the first two internodes, and typical stem structure becomes established only in the third. The stele in the transition

[1] I am indebted to Mr. R. PLATT of this Institution for making the necessary photomicrographs.

region is anomalous in structure, comprising both exarch and endarch bundles.

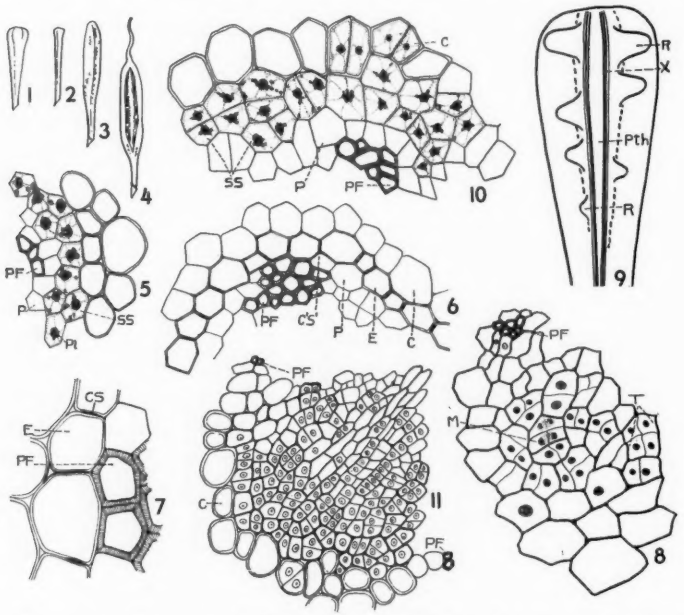
The variety Alaska resembles in essentials the variety Champion of England, so that only a brief outline of structure is necessary here. In the upper part of the third internode, the region of decapitation and treatment with which we are immediately concerned, stem structure is as a rule definitely established.

At this level the stem is still elliptical in outline. Pith and cortex are well developed. The fibrovascular system is made up of a central stele, two cortical fibrovascular bundles, and two fiber strands. Stele, cortical bundle, and fiber strand are each delimited by a starch sheath. The bundles of the central stele, six in number, two polar and two paired laterals, differ in extent and development, obvious in the number of xylem vessels, and they are separated by parenchyma rays. Both polar and paired lateral bundles are capped by groups of pericyclic fibers. The central bundles supply the leaf traces for the fourth and succeeding nodes. The cortical fibers follow the traces out into the rudimentary leaf blades, while the cortical vascular bundles, which are amphiphloic at this level, diverge into the stipules.

At the beginning of experimentation the seedling internode consists of primary tissue. Within twenty-four to thirty-six hours, however, some cambial activity is evident in the seriation of the intrafascicular differentiating xylem and phloem elements. The component cells of the tissues include epidermal, collenchyma, and parenchyma elements; starch sheath cells; pericyclic fibers only slightly thickened and pericyclic parenchyma; sieve tubes, companion cells and phloem parenchyma; spiral and annular, and later pitted elements constituting the xylem.

In unimbedded material the presence of starch in the innermost layer of the cortex serves to define the limits of the stele, the endodermis. In order to facilitate description, the term starch sheath is used for this limiting layer when the starch-containing plastids are in evidence, while the term endodermis indicates that differentiation of the Casparian strip has occurred. At the beginning of experimentation the starch sheath cells are vacuolated, and the starch-containing plastids are grouped as usual around the central nucleus (fig. 5).

Here and there plasmolysis typical of an endodermal cell is noted; that is, the protoplast, attached firmly to the radial walls, shrinks



FIGS. 1–11.*—Figs. 1–4, variation of form of swellings in auxin-paste and auxin-water series: 1, series A, typical swelling (164 hours); 2, series B (160 hours); 3, series C, 200 mg. per liter (160 hours); 4, series C, same with 1000 mg. Fig. 5, A.P. (24 hours) transection, swelling near tip; starch sheath begins to divide. Fig. 6, A.P. (164 hours) transection below swelling; endodermis complete and Casparian strip typical except at *C'*, *S'*. Fig. 7, A.P. (164 hours) transection, base of swelling; variation in extent of lignification of Casparian strip. Fig. 8, A.P. (48 hours) transection near cut surface; cell division in ray cells, mitotic figures, and "tetrad" appearance. Fig. 9, A.P. (140 hours) diagrammatic longitudinal section showing origin of root primordia at successive levels. Fig. 10, A.P. (81 hours) transection near base of swelling; division of starch sheath cells. Fig. 11, A.P. (92 hours) transection, young primordium.

* Abbreviations: A.P., series A. Seedling internodes treated with auxin paste for varying lengths of time, stated in hours. A.P. 2, series B. One week older than A, treated with paste in same way and grown under same conditions. W.S., series C. Same age as A, treated with aqueous solutions of auxin of varying concentrations. *C*, cortex; *CS*, Casparian strip; *C'*, *S'*, atypical lignification of strip; *E*, endodermis; *L*, limit of meristem cylinder; *M*, mitotic figure; *P*, pericycle; *PF*, pericyclic fiber; *Pl*, plastid; *Ph*, phloem; *Pth*, pith; *SS*, starch sheath; *T*, "tetrad" formation; *R*, root primordium *A*, *B*, etc.; *X*, xylem.

away from the outer and inner tangential walls. This condition is rare, however, and apparently bears no relation to cell position in

regard to rays or vascular strands. Later, in older seedlings (140–164 hours, control and treated) the starch sheath assumes the character of the endodermis of a root, and a Casparian strip is differentiated along the radial walls (fig. 6). The Casparian strip, however, is not visibly thickened as in some root endodermal cells. It is marked merely by a localized lignification of the middle lamella and of part of the primary wall (fig. 7). The secondary wall remains free of lignin material. Lignification is indicated with thionin, and tested with phloroglucin and HCl. Neither Sudan III nor di-methyl-amino-azo-benzene, however, indicates anything more than a faint possible trace of suberin in the modified region of the wall. The development of a functional endodermis in etiolated seedlings has been discussed at some length by PRIESTLEY (5). In *Pisum* the differentiation begins in the lowest internode and proceeds gradually upward toward the stem apex.

The differentiating phloem includes sieve tubes, companion cells, and phloem parenchyma. The sieve tubes are narrow, about one-third the diameter of the adjacent parenchyma cells, and they occur either singly or in groups of two or three. The pitted terminal sieve plates may be determined under oil immersion, and since the walls of the sieve tube stain deeply with fast green, they are distinguishable from the adjacent lighter companion cells.

ANATOMY OF SWELLINGS (SERIES A)

Tissues react to treatment with growth substances (1) by cell expansion (seen principally in the cortex and the pith); and (2) by cell division, notable in the inner cortex, endodermis, pericycle, ray parenchyma, cambial tissue, phloem parenchyma (primary and secondary), and xylem (to a slight extent).

Meristematic activity culminates in the production of root primordia. The seedlings collected at eight hour intervals give a connected picture of the development of the swellings and the included roots.

While development is a continuous process, it may for convenience be considered in four phases. These follow in succession, overlapping of course, but each characterized by a certain dominant activity.

This sequence, although general, is by no means inalterable, and a certain amount of individual variation occurs.

1. Swelling of parenchyma cells, particularly in the cortex and the pith, and infiltration of lanolin into intercellular spaces and spiral vessels (0–48 hours).

2. Formation of a meristem cylinder (16–72 hours).

3. Initiation and development of root primordia (56–164 hours).

4. Lignification of tracheids (116–164 hours).

1. SWELLING OF PARENCHYMA CELLS.—During the first twenty-four hours a definite expansion of the axis is observed which is due mainly to enlargement of the cortical cells. The latter, next to the cut surface, round off and may project as a raised rim around the non-extended vascular bundles and fibers. The parenchyma of the pith, the rays, and the parenchymatous elements of xylem and phloem react in essentially the same way but to a lesser degree. The net result is the rounding of the cut surface as a whole. The zone of maximum expansion in the present instance lies 1–2 mm. below the cut, but the level of this zone depends on auxin concentration. This fact is clearly seen in the aqueous auxin series C (figs. 1–4).

Enlargement of intercellular spaces accompanies cell expansion and allows of the physical penetration of the auxin paste into the tissues, which penetration may be seen by staining with Sudan III. In the first eight hours the intercellular spaces of the cortex are already blocked 3–4 mm. below the cut surface, and the same is true for the pith. Penetration keeps pace with the swelling, and in forty hours the narrow intercellular spaces of the outer cortex and the wider spaces of the inner cortex of the entire segment (1 cm. in length) are practically completely infiltrated. At the same time lanolin is drawn down into the cut spiral vessels to a depth of 7–10 mm. in the first eight hours. At the end of twenty-four hours most of the vessels in the 1 cm. segment are completely blocked (fig. 25).

Older seedlings were examined in order to determine the maximum depth of lanolin penetration. In the cortex this usually does not extend beyond the first centimeter, but in a few of the vessels the lanolin may reach a depth of 17 mm. Of the vessels so blocked, some are narrow, some wide in lumen, so that infiltration is not a function of vessel diameter. Metaxylem elements in general are unblocked,

but a few elements next to the cut surface, in seedlings treated for forty hours, contain intermittent lanolin plugs.

Penetration of intercellular spaces occurs in both control lanolin and in lanolin-auxin seedlings. As already stated, in the former no swelling of the cut internodes results.

2. FORMATION OF MERISTEM CYLINDER.—Active cell division is indicated: (a) by the presence of small, thin walled cells with relatively large nuclei, as in typical meristematic dividing tissue; and (b) by the position and relative thinness of newly formed cell walls in the larger vacuolating dividing cells. This is particularly obvious in the parenchyma cells of the cortex and the rays. In these tissues the outline of the parent cells is distinct, while the daughter cells within are grouped sometimes as tetrads, but more frequently in less regular formation (fig. 8).

Within twenty-four hours meristem activity is beginning near the surface. Twenty-four hours later the following tissues are involved in active division: inner cortex, starch sheath, pericycle, phloem parenchyma, ray tissue, the cambial region, and to a limited extent the marginal xylem parenchyma. The result is a hollow cylinder of meristem encircling the xylem and the inactive pith (fig. 26). This tapers downward into the still normal stem 5–8 mm. below the cut surface. At the base of the 1 cm. segment, therefore, the cambium functions normally and xylem and phloem elements differentiate at the usual rate, while above, in the region of swelling, differentiation does not keep pace with the active division of the meristem cylinder cells. The daughter cells in this region are shorter and wider than normal and eventually differentiate as tracheids and shorter phloem elements.

Cell division is not confined to the central stele, but occurs also in the cortical bundles, phloem parenchyma, endodermis and pericycle, and occasionally in the adjacent cortical cells.

Rapidity of cell division is occasionally emphasized in the parenchyma tissues by the presence of 2–4-nucleate cells.

A very slight trace of a potential cork cambium is indicated in the outer cortex and pith in a few cells immediately below the cut surface, but it remains inactive.

The formation of lacunae in the swellings is not unusual. A break-

down of the middle lamella occurs in the parenchyma tissue, usually along the inner face of the cortical bundles. Lacunae begin to develop about the same time that primordia originate (fig. 27).

3. INITIATION AND DEVELOPMENT OF PRIMORDIA.—In the oldest seedlings (164 hours) the root primordia are apparent to the naked eye in serial sections, and may be demonstrated in the intact shoot by staining with IKI. As a rule they arise in whorls, one to four at any level, the oldest next to the cut surface, the younger progressively lower (figs. 9, 29). Initials appear in the rays, except in the region of maximum swelling, where origin, distribution, and number are apt to be somewhat less regular. In transverse section only one primordium usually appears in exactly median section, while the other two or three are tangentially cut. Thus orientation of rootlets at any one level is actually spiral, not cyclic as would appear from a superficial examination. Rootlets in general pass horizontally outward through the cortex, but occasionally they bend first downward, then outward.

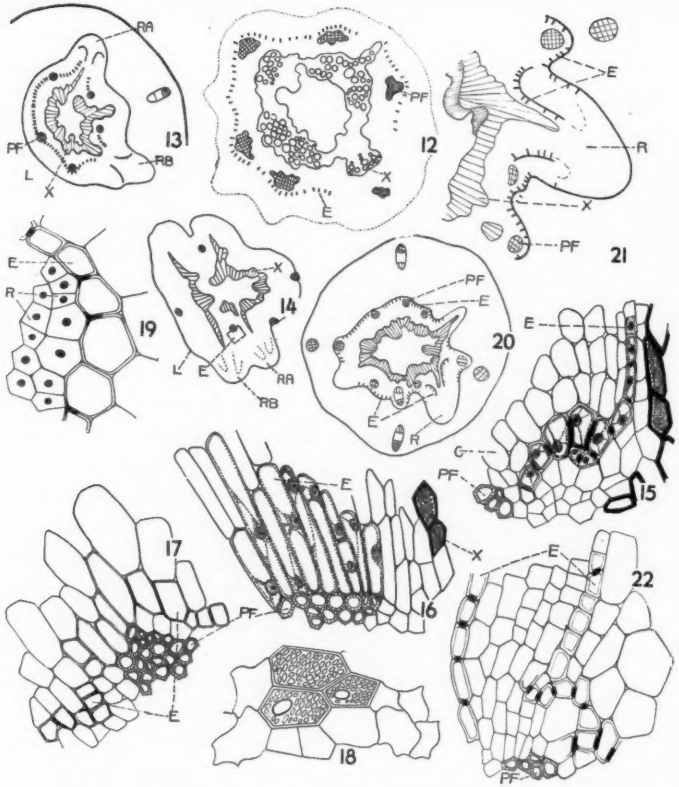
The development in time of the roots may be followed in material collected at stated intervals, and corroborated by the distribution in space of the primordia of different ages present in older swellings (116–164 hours). Origin and differentiation are intimately bound up with the formation of the meristem cylinder. In this connection sections of non-imbedded material stained with iodine are useful in following the distribution of the starch-containing cells. Near the lower limit of the older swellings, periclinal division is evident in the starch sheath cells, particularly across the ray (fig. 10). The plastids containing starch grains are distributed approximately equally to the daughter cells. Similar periclinal divisions appear next in the pericycle, and are followed immediately by anticlinal and irregular walls in both layers. Thereafter meristematic activity extends to the inner cortex. Root primordia are first distinguished as actively dividing cell groups, irregular in outline, near the outer margin of the rays. A primordium therefore is not distinctly traceable to pericycle alone, but arises in a complex of elements derived from inner cortex, endodermis, and pericycle.

Continued cell division results in the definition of a blunt root tip covered by an irregular sheath of starch containing cells (fig. 11), but the histogens are not defined at this stage. Elongation and dif-

ferentiation of the young root proceed in the usual way. Procambial strands give rise to stelar tissues, spiral vessels, and functional phloem elements. The latter develop in continuity with the axial phloem. Continuity between the water conducting tissues of axis and root is likewise established. Lignification of the ray cells at the base of the root gives rise to a central core of xylem, pitted tracheids, which connect laterally with the xylem of the axial vascular bundles.

In the lower internodes the change from typical starch sheath to typical endodermis, already described, is meanwhile effected. Demarcation of the endodermal region by lignification continues upward into the swelling. As might be expected, here the end result is somewhat different. The meristem cylinder, composite in origin, has differentiated by this time into cortex and stelar tissues. Intercellular spaces are absent in the stelar zone but abundant in the cortical, and are visibly blocked with auxin paste. (Since this cortical tissue originates from differentiation of the meristem cylinder, to clarify description it may be called meristematic, in contrast to normal primary tissue.) Differentiation of the endodermis, the boundary between stele and cortex, continues from the normal stem below, directly upward. Lignified Casparian strips differentiate on the walls of the innermost layer of this "meristematic" cortex. The development is gradual, so that in transverse sections at succeeding levels the endodermis appears as a complete or an interrupted ring (fig. 12). Lignification appears first across the ray arcs, and later across the pericyclic arcs. This lignification of the Casparian strip may be "typical," a narrow ring surrounding the cells, apparent therefore on the radial walls in transverse section; on the other hand it may be irregular in extent. Thus in transverse section the entire radial wall, and segments of the outer tangential wall, may react to phloroglucin and HCl. Occasionally an entire group of cells is affected, generally near the base of a root primordium (fig. 15). Intercellular spaces are always adjacent to lignified wall areas. The endodermis is interrupted by the developing roots, but because of its lignification, it may be followed from the axial cylinder outward, parallel with and external to the differentiating stele of the primordia. It is therefore in direct continuity with the differentiating endodermis of the young root (figs. 13, 15).

This appearance is strikingly different from that of an endodermis



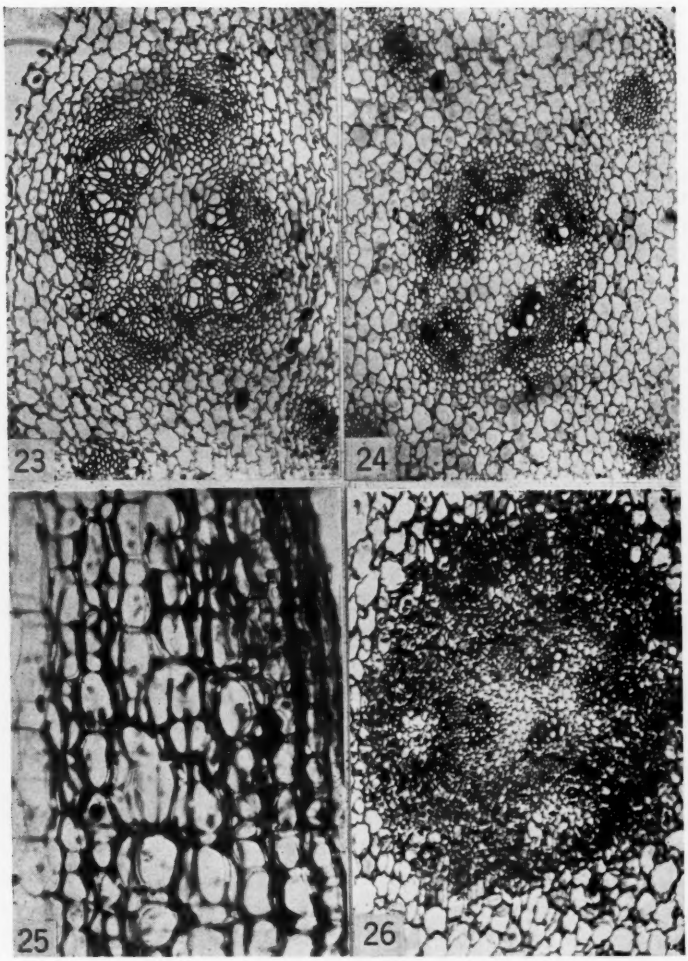
FIGS. 12–22.—Fig. 12, A.P. (164 hours) transection, base of swelling; endodermis not yet continuous. Fig. 13, A.P. (164 hours) transection, swelling with primordia *RA* and *RB:* axial endodermis continuous with developing endodermis of root *RA;* root *RB* cut tangentially. Fig. 14, A.P. (164 hours) transection, similar swelling cut at higher level; endodermis not yet present; two primordia *RA* and *RB;* for detail of region *E* see figs. 16 and 17. Fig. 15, A.P. (164 hours) transection at primordial base; axial endodermis continuous with developing endodermis of primordium; irregular lignification of "endodermal" group of cells (*cf.* fig. 13 root primordium *RA*). Fig. 16, A.P. (164 hours) detail of region *E*, fig. 14; radial elongation of endodermal cells. Fig. 17, A.P. (164 hours) transection near primordial base; radial elongation of endodermal cells near root base and irregular lignification in "endodermal" cell group. Fig. 18, A.P. (164 hours) lignification of pitted tracheids in ray and vessel-like perforations. Fig. 19, A.P.2. (160 hours) transection, detail of endodermis surrounding root primordium. Fig. 20, A.P.2. (160 hours) transection, primordium developing within endodermis. Fig. 21, A.P.2. (160 hours) detail of endodermis of axis and developing primordium of fig. 20. Fig. 22, A.P.2. further detail of same. (See footnote to figs. 1–11 for explanation of abbreviations.)

usually associated with root formation. In normal secondary root ontogeny the axial endodermis retains its identity, and by division and expansion keeps pace with the developing conical primordium. The endodermis of the young root meantime differentiates around the stelar cylinder and connects basally with the axial endodermal sheath. In the present instance the original starch sheath (or endodermis) is obliterated in cell division. A second functional endodermis appears after differentiation of the primordia and maintains continuity with the primary endodermis below.

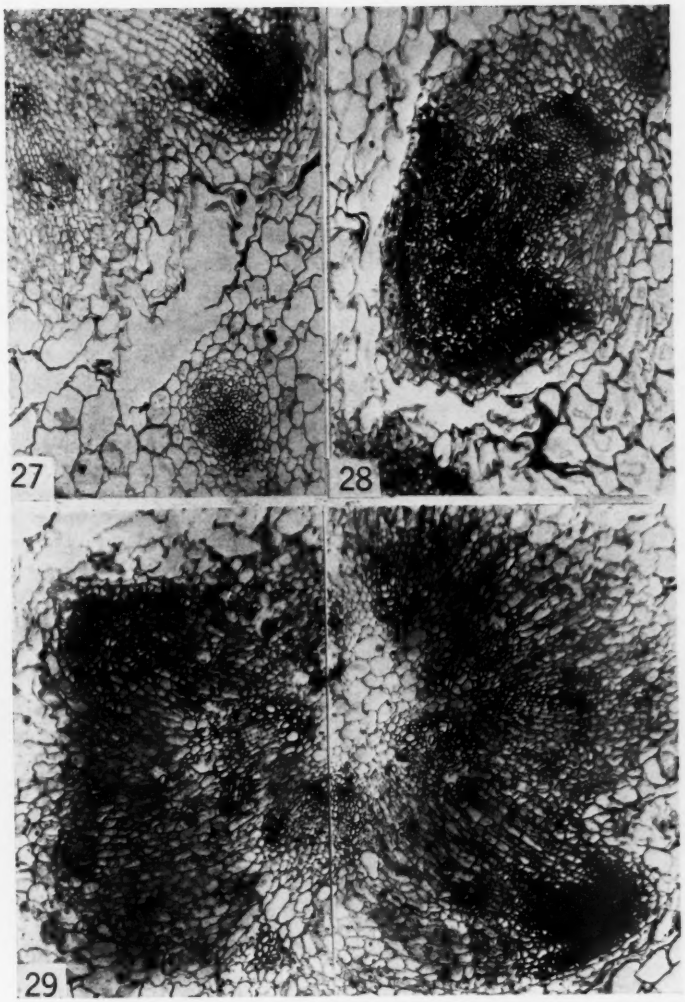
The endodermal cells usually are not exaggerated in size. Marked radial elongation, however, similar in appearance though not in ontogeny to that in *Phaseolus*, is occasionally observed. Root primordia may arise in adjacent rays at approximately the same level, and keep pace with each other in growth (fig. 14). Between two such bases lies a strand of primary pericyclic fibers and extra-pericyclic meristematic tissue, which from its position may be termed secondary endodermis. When the roots begin to lengthen rapidly, the secondary endodermal and adjacent cells expand in a radial direction to match the cells of the region of elongation of the young root (fig. 16). Endodermal cells farther removed from the root base may be similarly affected to a lesser degree. The radial walls of such cells may also become completely or partially lignified (fig. 17).

Root primordia originate also in connection with the cortical vascular bundles. Meristematic division begins in the internal phloem of the amphiphloic bundle, and in the adjacent endodermal and cortical cells the primordium with its root cap is gradually defined as before, and in this case is directed toward the center of the stem. During growth, however, it swings first tangentially and then vertically downward through the cortex. No primordia appear in connection with the cortical fiber strands, nor does cell division occur (fig. 28).

4. Lignification.—Within the limited time of experimentation (164 hours) the lignification of tissues is not particularly evident. Lignification of the ray cells in the intervals between root primordia is somewhat sporadic. The resultant tracheids are approximately isodiametric, or slightly elongated. The wall marking is pitted or reticulate, and both types of marking are occasionally present on the



FIGS. 23–26.—Figs. 23, 24, difference in vascular xylem development in treated and control seedlings: 23, A.P. (164 hours) transection below swelling, 1 cm. below cut surface; 24, control decapitated stem at same level (164 hours). Fig. 25, A.P. (48 hours) longisection, penetration of auxin paste, stained with Sudan III. Fig. 26, A.P. (56 hours) transection, meristem cylinder.



FIGS. 27–29.—Fig. 27, A.P. (90 hours) transection, lacuna. Fig. 28, A.P. (164 hours) transection, cortical primordium. Fig. 29, A.P. (164 hours) transection near apex of swelling; three root primordia, only one approximately median. (See footnote to figs. 1–11 for explanation of abbreviations.)

same wall. Vessel-like perforations are commonly seen in transverse section. Lignification appears about 100 hours after auxin treatment, and is not observed in any control (fig. 18).

Anatomy of swellings (series B)

The older internodes used in this series were decapitated and treated with auxin paste as before, and thereafter collected at intervals of 64, 112, and 160 hours. In the 64 and 112 hour specimens no root formation was indicated, and swelling in a few instances only was barely visible. Five of the sixteen seedlings allowed to grow for 160 hours developed small swellings and two to six roots. The swellings were generally split lengthwise along part of one side, and the root primordia were visible beneath or projected from the slit. The region of maximum swelling is 2 to 3 mm. from the cut surface and the tissue immediately adjacent to the latter 1 to 2 mm. is usually discolored and necrotic.

The chief points of comparison between this and the previous series are: (1) Meristematic activity is very much less in all tissues. (2) The parenchyma tissue is much less plastic, with the result that splitting of the cortex occurs during cell division and root formation. (3) The pith is very frequently entirely lignified. (4) The behavior of the endodermis forms the most interesting point of comparison (figs. 19–22).

As was expected, the Casparian strips of the endodermis were lignified in the lower internodes up to the base of the swelling. Within the latter the endodermis is more or less complete at successive levels. Whether it is unlignified or lignified as to Casparian strips, however, it maintains its identity as an individual layer, and is traceable around the developing root primordia (fig. 19). Periclinal cell division does not occur. As the primordium develops, a stelar endodermis is differentiated within it which connects basally with the axial endodermis, thus simulating the normal condition seen in secondary root development (figs. 20, 21, 22).

Anatomy of swellings (series C)

The seedlings in this series were grown as in series A. When the leaf of the fourth node was apparent they were decapitated as

before, but the cut internodes were then inserted in aqueous solutions of auxin of varying concentration for forty-eight hours. Thereafter the seedlings were allowed to continue growth as before. The results are summarized in table 1.

It is seen from the table that the outline of the swelling and the number of roots depend upon the concentration of the auxin solution. The swelling varies in form from the blunt club seen in the weakest solution to the long ellipsoid ending in 2 cm. of shriveled necrotic axis, of the strongest solution (figs. 3, 4).

TABLE 1

Concentration in milligrams per liter	Distance of maximum swelling from cut surface (mm.)	Length of swelling (cm.)	Number of rooted swellings	Number of roots per swelling
100	1–2	1–1.5	4/10	1–13 roots 3–3–4 roots
200	2–3	1.7–2.5	10/10	All 12–18
400	4–5	2.5–3	10/10	All 60–90
1000	200	2.5–3	10/10	All 80–120

The anatomy of swelling and of root formation is essentially the same as in the paste series.

The meristem cylinder is differentiated as before, but the intercellular spaces are larger, resulting in a looser tissue. In the weakest solution meristem activity does not culminate in root formation. The intense development of roots seen in the strongest solution causes a vertical splitting of the stem. This region of the stem affected, 2 cm. below decapitation, has practically ceased to elongate and is therefore less plastic than the younger part of the internode.

The behavior of the endodermis is essentially similar to that in series A. As a starch sheath it becomes obliterated by intense meristem activity. By lignification of Casparian strips it is redefined in the region of the swelling.

From a comparison of series B and C it appears that the endodermis loses its capacity for division sooner than does the pericyclic and ray tissue.

Discussion

In one of the earliest surveys on root anatomy, JANCZEWSKI (3) outlines the various types of development seen in the primary and secondary roots of higher plants. The Leguminosae, including *Pisum sativum*, differ from the majority of other dicotyledons, in that secondary roots originate from three cell layers (pericycle, endodermis, and inner cortex) and the histogens are differentiated comparatively late (current terminology is used here in place of the terms pericambium, etc.).

VAN TIEGHEM and DOULIOT (6) later rejected JANCZEWSKI's findings in reference to the Leguminosae, and attributed the entire primordium of the secondary root to division of pericyclic cells. A temporary digestive pocket or sheath only, originates from the outer tissues, the endodermis, and the cortex. Adventitious roots, arising from a hypocotyl base, are essentially similar in origin to secondary roots. The primordia of adventitious and secondary roots, while both endogenous, necessarily vary somewhat in their method of differentiation. In the present instance, where etiolation induces the formation of a root endodermis in the stem, the situation becomes more complex.

The root primordia of *Pisum* vary in origin according to the age of the internode and the concomitant development of the endodermis. In the younger seedlings (series A) the primordium is derived from a complex of tissues—endodermis, inner cortex, and pericycle. It thus resembles the original description given by JANCZEWSKI. In the older specimens (series B), whether or not the Casparian strip is lignified, the endodermis maintains its identity. The primordium is therefore derived from the pericycle as VAN TIEGHEM has indicated. No comparison of JANCZEWSKI's and of VAN TIEGHEM's accounts of root initiation in the normal plants has been made at the present time.

In recent papers KRAUS (4) and BORTHWICK (1) and their collaborators trace the root primordia to a complex group of meristem cells, not to a single definite layer. The younger seedlings of *Pisum*

therefore resemble fundamentally the seedlings of *Phaseolus* and *Solanum*.

Certain differences are observed, however, in the specific tissue reactions of the three genera. The endodermis of *Phaseolus*, for instance, "is highly responsive to indoleacetic acid. Within 168 hours, cells 5 cm. below the point of application are highly meristematic" (4). In etiolated seedlings of *Pisum* the stimulus to active cell division does not in any case extend below 6–8 mm. in 164 hours. There is thus a marked difference in the reaction of the endodermis cells in the two genera. Whether this reaction is specific or is conditioned by the etiolation of *Pisum* is undetermined.

Another instance of marked difference in activity is seen in the xylem parenchyma and in the pith. In *Phaseolus* both these tissues become meristematic, whereas in *Pisum* the pith cells are inactive and the xylem parenchyma practically so.

The positions of the primordia are also contrasted. Whereas in *Pisum* the root initials occur most commonly in the rays, in *Phaseolus* and also in *Solanum* they appear in the phloem. In *Solanum* a double ring of root primordia is developed in definite relation to the phloem strands of the bicollateral bundles.

It is clear therefore that the general reactions of living stem tissues to auxin so far established, namely, cell expansion, meristematic activity, tumor formation, and root development, occur in *Pisum* as in other seedlings previously described. Differences in detail appear in the specific reactions of individual tissues.

Two general conditions essential for the initiation of meristem activity are noted by PRIESTLEY (5): the presence of a blocking surface and a source of food material. The penetration of lanolin (control) and lanolin-auxin paste (experimental) into the cortical intercellular spaces suggests such a possible barrier, and a food supply is available in the adjacent phloem. The penetration of lanolin alone, however, does not induce cell division or swelling.

Attention has been called to the transformation, during etiolation, of the limiting layer of the cortex from a starch sheath to an endodermis with characteristic Casparian strips. An endodermis is structurally a barrier layer. Since in series A lignification of the Casparian strip does not precede differentiation of the root primordia, the

endodermis does not function in this connection. In series B, on the other hand, the primordial development occurs within the endodermis, as in the "typical" origination of a secondary root.

The vertical distribution of the root primordia has been commented upon. There is no obvious anatomical differentiation to account for their initiation. No unusual condition of intercellular spaces, no variation in lanolin infiltration, no change in endodermal structure is detectable when and where the root initials are first distinguishable. The appearance of primordia at definite vertical intervals indicates a periodic recurrence of the conditions and factors necessary for root formation. The solution of this problem depends on further physiological experimentation.

There is a lack of specificity of tissues. A generalized meristem cylinder arises from division of inner cortex, endodermis, pericycle, and other cells. From this apparently homogeneous tissue there are later differentiated such distinct elements as (1) endodermal cells with Casparian strips in a continuous cylinder one cell thick; (2) characteristic pitted tracheids, approximately isodiametric, in rays, root bases, and sporadically in inner cortex; and (3) parenchymatous elements of inner cortex and phloem.

Summary

1. Three series of etiolated seedlings of *Pisum sativum* were grown, decapitated in the third internode, and treated with auxin paste or with aqueous solutions of auxin. The treated seedlings were collected at eight hour intervals and observed for 164 hours. Controls were grown under exactly similar conditions.

2. The general results of auxin treatment, swelling and root formation, vary in extent in relation to seedling age and auxin concentration.

3. The form of the swelling varies in reference to auxin concentration.

4. Four phases of growth are recognized during the formation of a swelling, each characterized by a dominant activity: (a) expansion of parenchyma cells and infiltration of auxin paste into intercellular spaces (0–48 hours); (b) formation of a meristem cylinder from division of inner cortex, starch sheath, pericycle, and phloem paren-

chyma tissue (16–72 hours); (c) initiation and elongation of root primordia (56–164 hours); (d) lignification of tissues (116–164 hours).

5. The root primordia vary in origin in younger and older stems. In younger seedlings the identity of the endodermis is lost in the meristem cylinder complex. The root primordia are traceable to a group of ray cells within this complex. In older seedlings the endodermal cells do not divide and the primordia are therefore pericyclic and intra-pericyclic in origin. The endodermis is traceable as a primordial sheath. Casparian strips may or may not be lignified.

6. The roots arise approximately in whorls at successive levels on the stem. There is no visible anatomical differentiation to account for this distribution.

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GROWTH AND TROPIC RESPONSES OF EXCISED AVENA COLEOPTILES IN CULTURE[1]

GEORGE S. AVERY, JR., AND CARL D. LA RUE

(WITH SIX FIGURES)

Introduction

If the *Avena* coleoptile is removed from the seedling and transferred to a suitable culture medium, to what extent is it able to grow and utilize food and growth hormone artificially supplied to it? To what extent does it possess reserves of food and growth hormone at the time of excision? Is growth hormone really necessary for growth?

Most previous experiments involving growth of the *Avena* coleoptile and its response to various stimuli have been done with the intact seedling, and from this work have come two views: (1) that the tip of the coleoptile is the center of hormone synthesis in the seedling; and (2) that when this tip is decapitated, the upper end of the coleoptile stump, after approximately 2.5 to 3 hours, begins to function as a new physiological tip, producing growth hormone and enabling the stump to renew its growth.

Evidence is gradually accumulating that these views are in need of revision. For example, SÖDING (**8**) discovered that physiological regeneration took place to an equal extent whether one or more millimeters of the coleoptile tip were removed. This suggests that a precursor or even the auxin itself must be coming from another part of the seedling. POHL (**6**) reported that the coleoptile tip was not the center of hormone synthesis, but that it dispersed substances conveyed to it from the endosperm. POHL also added auxin-a to the seed; it was transported to the coleoptile where it stimulated growth. HEYN (**3**) found that in cut-off cylinders (segments) phys-

[1] Contribution from the Department of Botany, Connecticut College, and no. 664, Papers of the Department of Botany, University of Michigan. A part of this work was done at the University of Michigan Biological Station.

iological regeneration does not occur; however, when BONNER (**2**) immersed such cylinders in solutions of varying concentrations of growth hormone, growth was promoted at lower but retarded at higher concentrations.

The work of JOST and REISS (**4, 5**) has shown that segments 20 mm. in length (cut from coleoptiles 2–4 cm. long) undergo appreciable elongation in the first twenty-four hours after excision, the extent of elongation depending upon the concentration of indole(3)-acetic acid in the culture solution, and whether the tip or basal end was immersed. From the work of SÖDING, and POHL in particular, it seems clear that in the normal seedling the growth hormone, or a precursor, moves upward from the endosperm and cotyledon through the vascular system to the tip of the coleoptile. From the tip it is dispersed downward through the coleoptilar tissues, presumably stimulating growth as it goes.

In the experiments reported here we have excised the young coleoptiles from their seedlings, thus removing their normal supply of food, water, minerals, etc., and cultured them in various ways.

Material and methods

Seeds of *Avena sativa* var. Victory (Svalov) were disinfected in a solution of calcium hypochlorite, transferred to sterile petri dishes containing moist filter paper, and germinated at 20°–25° C. When the coleoptiles were 3–12 mm. in length, they were excised from the developing seedlings and grown in both agar (under aseptic conditions) and liquid cultures (fig. 1). Certain cultures were supplied with sucrose or fructose, White's nutrient solution but without yeast (**10**), and indole(3)acetic acid; others were grown on pure agar or in distilled water. All cultures were grown in darkness at 20°–22° or 25°–26° C., and examined only in phototropically inactive light. Certain excised coleoptiles growing in culture were decapitated and then capped with agar blocks containing various combinations of mineral nutrients, sucrose, and indole(3)acetic acid; others were grown in an inverted position, with the decapitated distal end immersed in the solution (experiments without success on coleoptiles of the ages used).

Investigation

GROWTH HORMONE CONCENTRATIONS IN TIPS OF COLEOPTILES OF DIFFERENT AGES, FROM NORMALLY GROWING SEEDLINGS

One to 1.5 mm. tips of coleoptiles 2–47 mm. in length were removed, passed through a thin film of 5 per cent gelatin on a glass slide, and applied unilaterally to decapitated test coleoptiles. The first foliage leaf had not yet burst through the tips of any of the oldest coleoptiles tested. The results are plotted in figure 2. The

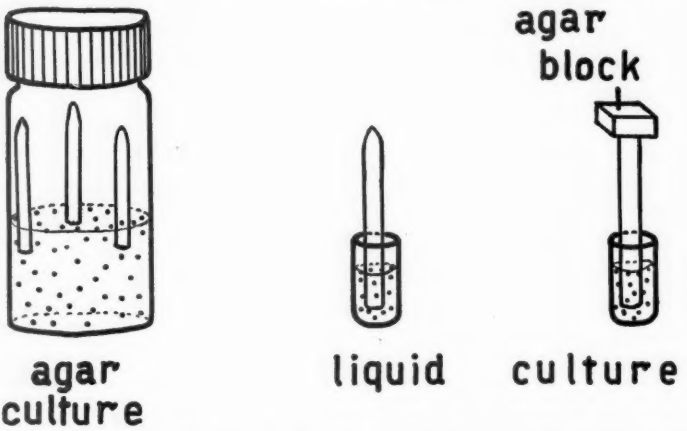

FIG. 1.—Methods of culture. The small glass containers used for liquid culture were supported in racks of 12 each. See table 2 in reference to figure on right.

tips of very young coleoptiles contained relatively low concentrations of growth hormone (see also WENT and THIMANN, 9). The most rapid increase in concentration occurred between the 2 and 6 mm. stages; this is correlated with the peak period of cell multiplication (1). No further appreciable increase was detectable after the coleoptile was 1 cm. in length although a peak content occurred near the end of the growth period.

From the preceding it is evident that tips from coleoptiles 3 mm. or less in length, excised for growth in culture, contain approximately one-half the hormone present in tips from coleoptiles 5–6 mm. or larger. If the hormone is the limiting factor for the growth of excised coleoptiles, those 6 mm. or more in length at the time of

excision should undergo a proportionately greater increase in length than those of approximately 3 mm. length, provided other factors are not limiting. The experiments which follow show this not to be the case.

Growth of excised coleoptile as an independent organ in agar culture

Influence of size of coleoptile at time of excision, and of various combinations of nutrient materials.—Two principal sizes of coleoptiles were used in this experiment in order to determine: (1) relationship of hormone content of tip at the time of excision to later growth of coleoptiles in culture; and (2) relationship between number of cells present at the time of excision (that is, the beginning reserve) and subsequent growth in length of excised coleoptiles.

Comparison of figures 2 and 3 indicates what at first glance appears to be a direct correlation between hormone content of tip and subsequent growth in culture. Comparison of percentage increase in length, however, reveals that coleoptiles in the series 2.6 to 2.7 mm. long at the time of excision, and with full nutrition, increased in length 434 per cent in the eight day culture period, whereas those with similar nutrition in the series 5.1 to 5.4 mm. at the start increased only 243 per cent. Similar relationships hold in the other series. Actually therefore there is an inverse relationship between hormone content of the tip at the time of excision and subsequent growth of the coleoptiles in culture. Such evidence suggests the view that growth hormone is not limiting for growth of coleoptiles in culture.

It has been shown previously that cell division goes on rapidly in the parenchyma of young coleoptiles (1): the number of cells in the subepidermal layer (median longisection) of a 2.5 mm. coleoptile approximates 90, that of a 5.0 mm. coleoptile, about 145. The growth curves of the coleoptiles in the series 2.71 mm. at the beginning of the experiment (fig. 3), grown on agar with White's solution, fail to attain the final length of those with similar nutrition of 5.3 mm. average length at the beginning of the experiment. With no further increase in numbers of cells, elongation of the cells already

existing at the time of excision (90 and 145 respectively) would account for the differences in final length. Cell division apparently did not continue under these culture conditions. However, even under such conditions of nutrition with sucrose lacking, the shorter excised coleoptiles continued to elongate for five days in culture.

The presence of sucrose in the nutrient medium enabled coleoptile growth to continue for seven and eight days, but again the coleoptiles which were short at the beginning of the experiment never attained the final length of those which were longer at the beginning, although

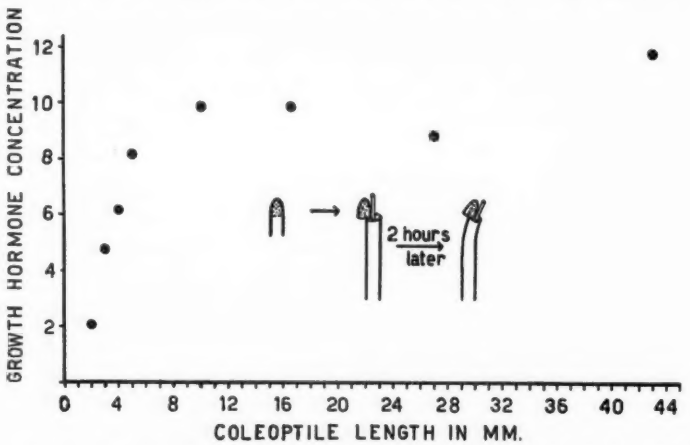

FIG. 2.—Concentrations of growth hormone in coleoptile tips of normal seedlings of different ages. Each point represents average of 48 coleoptile tips tested. Seedlings grown and tips tested in darkroom at 26° C. and 88–90 % relative humidity.

their percentage increase in length was much greater for agar and sucrose, 404 against 267 per cent; full nutrition, 434 against 243 per cent. A few cell counts in longisections of the coleoptiles showed a greater proportional increase in the number of cells in the short set than in the long (final average cell length was approximately the same in both sets). Hence, with sucrose in the culture medium, short coleoptiles with less reserve make a proportionately greater growth by adding appreciably to their reserve.

To determine further the effect of nutrition on subsequent growth of coleoptiles of different ages at the time of excision, three addi-

tional series of experiments were set up (fig. 4). Growth of these older coleoptiles was substantially completed at the end of four days in culture. Hence the older the coleoptile at the time of excision, the less the growth response in culture.

INFLUENCE OF INDOLE(3)ACETIC ACID SUPPLIED THROUGH CULTURE MEDIUM.—Six series of coleoptiles, thirty-six in each series, were excised from seedlings when they had reached a length of 4 mm.

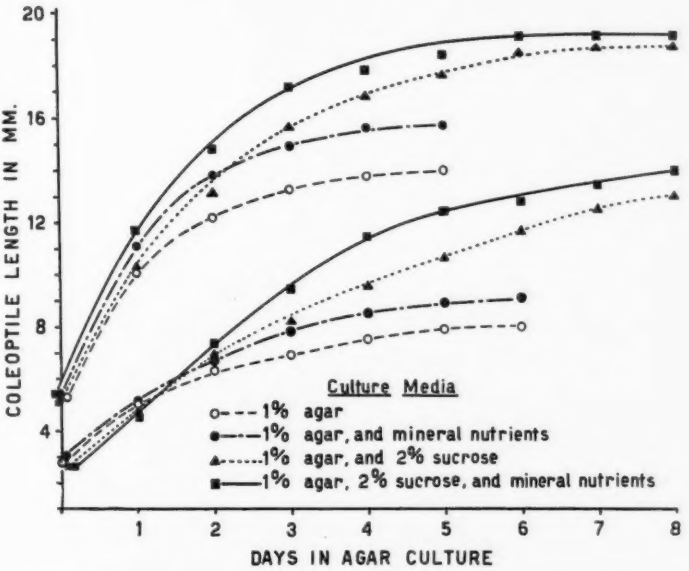

FIG. 3.—Growth of coleoptiles in agar culture with varying conditions of nutrition. Coleoptiles were of two different age groups at time of excision: 2.6–2.7 mm. and 5.1–5.4 mm. in length. There were 12 coleoptiles in each series. Darkroom temperature, 20°–22° C. See fig. 1 for method of culture.

Their cut bases were inserted into 1.5 per cent agar containing mineral nutrients, 2 per cent sucrose, and different concentrations of indole(3)acetic acid. They were allowed to grow for eight days in a darkroom at 24° C. The results are shown in figure 5.

In all the concentrations tested, indole(3)acetic acid showed a depressing effect on growth in length. This effect was noticeable in

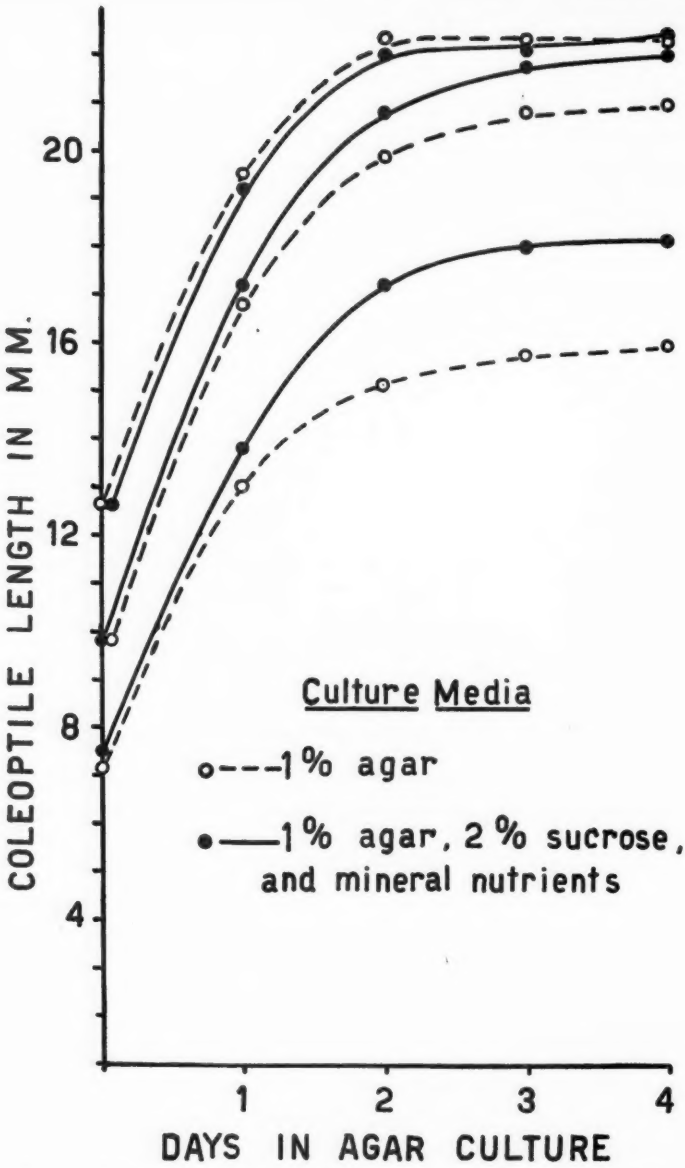

FIG. 4.—Continuation of fig. 3. Coleoptiles were of three different age groups at time of excision. Note influence of size of coleoptile at time of excision upon subsequent response to nutrition during growth in culture. Darkroom temperature, 26° C.

dilutions as great as 1 : 5,000,000, and higher concentrations retarded growth to correspondingly greater degrees. Computations show that the apparent differences in growth shown in figure 5 are statistically significant. Is it possible that physiologically similar substances produced in the normal seedling have no causal effect on growth in length of the coleoptile?

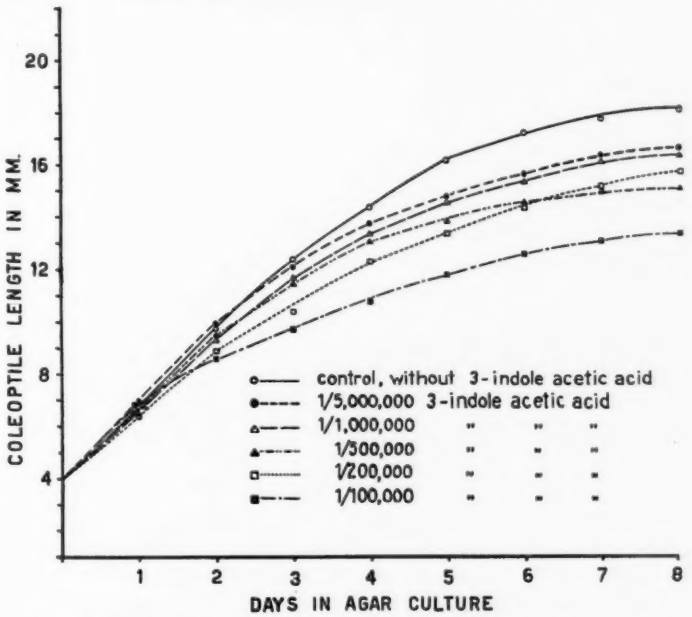

FIG. 5.—Influence of indole(3)acetic acid (supplied through culture medium) upon growth of coleoptiles in culture. Darkroom temperature, 24 °C.

The lowest concentration of indole(3)acetic acid used in this experiment (1/5,000,000) produces about 15° curvature when applied unilaterally to decapitated coleoptiles. WENT and THIMANN (9) recorded approximately 21° curvature for the same concentration. The substance which produces curvature proportional to concentration when applied to the tips of decapitated seedlings also causes depression of growth proportional to concentration when applied at the bases of excised coleoptiles.

INFLUENCE OF pH OF CULTURE MEDIUM.—Experiments involving the growth of excised coleoptiles on agar adjusted with hydrochloric acid (not buffered) to pH 4, 5, 6, and 7 yielded no appreciable nor constant differences.

GROWTH OF EXCISED COLEOPTILE AS AN INDEPENDENT ORGAN IN LIQUID CULTURE

In order to make certain that an adequate supply of water was available to the artificially cultured coleoptiles, liquid cultures were set up. The base of each coleoptile was placed in a small glass culture cup, as illustrated in figure 1, and the solutions were changed daily at the time the coleoptiles were measured. In order to be certain that liquids could enter the coleoptiles readily, about 0.2 mm. was cut from the base of each at the time of changing the culture solution. To test the entrance of liquids, forty-eight excised coleoptiles were placed in culture cups containing dilute solutions of fast green, green ink, etc. In every instance the dye had ascended to the tip of the coleoptile within a two hour period, thus leaving no doubt that materials present in the culture solution were being taken up by the coleoptiles.

INFLUENCE OF SUCROSE IN CULTURE SOLUTION.—In order to determine whether certain concentrations of sucrose might effectively promote growth of older excised coleoptiles in liquid culture, culture solutions containing 2 to 10 per cent sucrose were set up (table 1). The coleoptiles were 9.5 to 12.5 mm. in length (in agar culture, 2 per cent sucrose had a slightly favorable effect on subsequent growth of coleoptiles excised when approximately 10 mm. in length), and therefore well past the main period of cell division at the time they were excised. Growth of the coleoptiles in the lower concentrations of sucrose (2 to 4 per cent), although not so good as the controls over the first twenty-four hours, gave closely parallel results; successively higher concentrations resulted in proportionate decreases in growth. However, higher concentrations of sucrose extended the length of the growth period by one to two days, and gave somewhat better growth on the fourth and fifth days. Similar experiments, using fructose, yielded results of the same order. Hence the conclusion is that in liquid culture, as in agar culture, the addition

of sugar to the culture medium was not advantageous to the growth of older coleoptiles.

INFLUENCE OF CAPPING DECAPITATED COLEOPTILES WITH AGAR BLOCKS CONTAINING VARIOUS NUTRIENTS AND INDOLE(3)ACETIC ACID. —In order to test the influence on growth of agar blocks containing White's solution with and without sucrose, coleoptiles again were

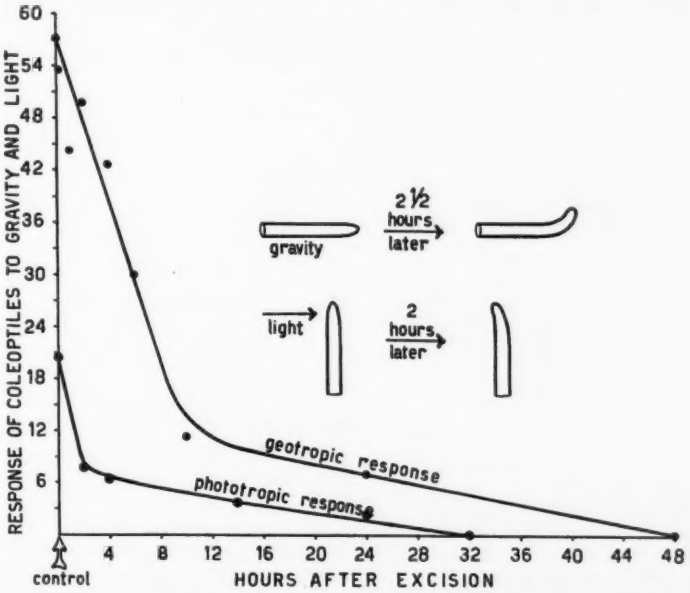

FIG. 6.—Geotropic and phototropic response of excised coleoptiles. Each point on the graph represents average of 12 coleoptiles. Coleoptiles were kept in an upright position (from time of excision through period of stimulation) on moist filter paper in vertically placed petri dishes. Geotropic stimulation for 2½ hours before recording curvature. Unilateral light stimulus from 40-watt Mazda lamp at a distance of 15 feet for 2 minutes; curvature recorded 2 hours later.

used which were past the cell division phase of their development; they were 12.5–13.7 mm. in length after decapitation, at the time of placing in culture cups in distilled water. The agar blocks were placed on the apices of the decapitated coleoptiles (fig. 1). There were no appreciable differences in growth, whether the coleoptiles were capped with blocks made up with distilled water only, or with White's solution, or White's solution with sucrose or fructose.

In order to determine the influence on growth of agar blocks containing indole(3)acetic acid, coleoptiles 18–19 mm. in length were excised, placed in culture cups in distilled water, and decapitated to

TABLE 1

GROWTH OF EXCISED AVENA COLEOPTILES IN LIQUID CULTURE WITH VARYING CONCENTRATIONS OF SUCROSE. TWELVE COLEOPTILES IN EACH SET, RANGING IN LENGTH FROM 9.5 TO 12.5 MM. AT BEGINNING OF EXPERIMENT. DARKROOM TEMPERATURE 26° C.; RELATIVE HUMIDITY 88–90 PER CENT

CULTURE MEDIUM	DAILY GROWTH INCREMENT IN PERCENTAGE OF PREVIOUS DAY'S LENGTH					
	1	2	3	4	5	6
Distilled water	61	12	7	3	0	0
Sucrose 2%	48	13	9	2	0.5	0
3%	46	.....	10	4	2	0
4%	46	11	10	1	2	0
5%	35	14	13	9	3	0.5
6%	25	11	9	6	2	0.5
7%*	19	.....	5	4	2	0.5
8%	18	11	4	5	4	0
9%	16	11	7	10	4	0
10%	13	14	6	7	2	0

* Growth continues through 9th day in cultures with 7–10 per cent sucrose; best growth in 8–9 per cent sucrose.

TABLE 2

INFLUENCE OF INDOLE(3)ACETIC ACID ON GROWTH IN LENGTH OF AVENA COLEOPTILES; APPLIED IN 1.5 PER CENT AGAR BLOCKS TO DISTAL CUT SURFACE OF COLEOPTILE STUMP. RESULTS GIVEN IN PERCENTAGE INCREASE OVER CONTROLS (PURE AGAR 1.5 PER CENT) AFTER 24 HOURS. TWELVE COLEOPTILES IN EACH SET. DARKROOM TEMPERATURE 26° C.; RELATIVE HUMIDITY 88–90 PER CENT

COMPOUND	CONCENTRATION			
	Dilution: 1:3,200,000 γ per liter: 312	1:800,000 1250	1:20,000 50,000	1:5,000 200,000
Indole(3)acetic acid	10.4	13.4	18.3	13.4

16 mm. in length. The agar blocks were placed on the apices of the decapitated coleoptiles, with resulting elongation appreciably greater than the controls (table 2).

For straight growth of coleoptilar stumps still attached to their

seedlings but with indole(3)acetic acid as applied in this experiment, SCHEER (7) found a 31.9 and 25.9 per cent increase in length over the controls for 1:20,000 and 1:5000 respectively.

GEOTROPIC AND PHOTOTROPIC RESPONSE OF EXCISED COLEOPTILES

That excision has a marked effect on the capacity of coleoptiles to respond to the stimuli of gravity and light is clear from figure 6; no attempt was made to adjust the periods of stimulation so that the initial magnitude of curvature would be the same. In the phototropic experiment the coleoptiles were 20 mm. long at the time of excision, while in the geotropic experiment they ranged from 30 to 35 mm. in length. The capacity to respond fell to approximately 20 per cent of that of the controls in 10–14 hours after excision. Contrasted with this is a drop to 55–65 per cent (of that of the intact coleoptiles) in the output of growth hormone by the tips of such excised coleoptiles after twelve hours' excision.

Summary and conclusions

1. Determinations were made of the hormone concentration in the tips of normal coleoptiles from seedlings of different ages; the tips of very young coleoptiles contain relatively low concentrations and the most rapid increase in concentration occurs while coleoptiles elongate from 2 to 6 mm. After the coleoptile is 1 cm. long, hormone content in the tip remains at a relatively constant level on to the end of the growth period.

2. Coleoptiles of different ages were excised and grown aseptically on 1 to 1.5 per cent agar, agar with sucrose, and agar with sucrose and mineral nutrients; indole(3)acetic acid was supplied through the culture medium in certain experiments. Coleoptiles were grown also in liquid cultures. The coleoptiles grew equally well on agar adjusted (not buffered) to pH 4, 5, 6, or 7.

3. Coleoptiles excised at a length of 3 mm. or less failed to reach the length attained by coleoptiles grown under the same conditions which were more than 5 mm. long when excised. However, the 3 mm. coleoptiles showed a far greater percentage increase in length than those 5 mm. long at the start of the experiment (although the initial hormone concentration in the tip was much lower). The addi-

tion of sucrose and mineral nutrients had a markedly beneficial effect on growth of such coleoptiles in culture; when 12 mm. or more in length, nutrient substrate had no effect on growth in agar culture. Hence the older the coleoptile at the time of excision, the less the growth response in culture.

4. Low concentrations of sucrose in the liquid culture medium gave better results than high, although high concentrations prolonged the growth period somewhat.

5. The addition of indole(3)acetic acid to the nutrient agar in which excised coleoptiles were grown retarded growth in length. This retardation was proportional to the concentration of the indole-(3)acetic acid.

6. Decapitated excised coleoptiles capped with agar blocks containing indole(3)acetic acid showed significant length increases over the controls.

7. Excised coleoptiles gave geotropic and phototropic responses of rapidly decreasing magnitude over a twenty-four hour period. The capacity to respond fell to approximately 20 per cent of that of the controls ten to fourteen hours after excision; meanwhile hormone concentration in the coleoptile tips fell only to 55-65 per cent of that of the controls.

8. In conclusion, it is clear: (1) That growth hormone secreted by the coleoptile tip (as judged by concentration in the tips of young and older coleoptiles) has no helpful relationship to growth of coleoptiles in culture. The smaller the coleoptiles at the time of excision the greater their percentage increase in length in culture, in spite of their relatively lower content of hormone at the time of excision. (2) That growth of coleoptiles excised when approximately 3 mm. in length continues in agar culture (at 20°–22° C. and with suitable nutrition) for a period of eight days, or more.

9. In no instance, with present methods, have we found detectable concentrations of growth hormone in the tips of coleoptiles more than forty-eight hours after excision. If the substance is truly a necessity for coleoptile growth, the concentrations at which coleoptile elongation is stimulated are very very low, and growth must continue for six days at such undetectably low concentrations. The evidence makes another explanation equally plausible—that growth

hormone is not a necessity for elongation of the coleoptile. Such an interpretation would not mean that growth hormone cannot stimulate growth of the coleoptile, for indeed it can, as illustrated throughout the voluminous literature on this subject, and shown again in table 2 of this paper. But nowhere has it been shown beyond question, in our judgment, that the hormone must be present if growth in length is to occur. Hence we suggest here a probable difference between growth stimulating effects of such physiologically active substances and their necessity for length growth. We regard as doubtful the view that growth hormone is a necessity for growth; that it can catalyze growth is quite another matter.

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RELATION OF ENVIRONMENT AND OF THE PHYSICAL PROPERTIES OF SYNTHETIC GROWTH SUBSTANCES TO THE GROWTH REACTION

DAVID M. BONNER

(WITH SIX FIGURES)

Introduction

A molecule must possess at least two general characteristics to function as an auxin or plant growth hormone. In the first place it must be transportable within the tissues of the plant; in the second place it must be capable of taking part in the chain of processes leading to cell elongation. In the pea test (**10**) longitudinal transport plays no part, and hence it is possible with the aid of this test to study the primary growth activity (**8**) of an auxin. A more detailed investigation of the reactions of a number of auxins in the pea test has been carried out, and is reported in this paper.

It was noted by KÖGL and HAAGEN SMIT (**4, 5**) while isolating pure auxin, that in testing the extracts in the *Avena* test it was of advantage to add small amounts of potassium chloride, and of acid. DOLK and THIMANN (**2**) investigated this same phenomenon while studying the nature of the growth substance, indole(3)acetic acid, produced by *Rhizopus sinuus*. This substance, in a concentration which showed slight activity in the *Avena* test when buffered at pH 7.0, was highly active when buffered at pH 5.0. These are apparently the first experiments in the field of plant hormones demonstrating the effect of the pH of the external solution on the activity of a growth promoting substance. Since that time (1931–32) there have appeared numerous papers dealing with this subject. One main hypothesis has developed as to this effect of acid on growth. J. BONNER (**1**), working with the *Avena* coleoptile, obtained a curve closely resembling the dissociation curve of a weak acid by plotting degrees acid curvature in his *Avena* test against the pH of the external solution. From this and further data he postulated that the action of acid on growth is through its direct action on auxin; and that auxin,

an organic acid, was active only in the non-dissociated state. A study of growth substances with different activities possessing different pKs (the negative logarithm of the dissociation constant) should thus give an insight into whether the difference in activities is due to a change in the essential molecular structure, or due only to a difference in physical properties.

Methods

The method of assay used was the pea test as formulated by VAN OVERBEEK and WENT (9). The indole(3)acetic acid (melting point 164° C.) used was kindly supplied by Merck, and the other growth substances used were either synthesized or purified by recrystallization by Dr. J. B. KOEPFLI. All pH measurements were made electrometrically, a glass electrode being used in a majority of the measurements. The buffer solutions were mixtures of (1) disodium hydrogen phosphate and potassium dihydrogen phosphate, (2) disodium hydrogen phosphate and citric acid,[1] or (3) potassium acid phthalate and either hydrochloric acid or sodium hydroxide. The particular buffer mixture used played no role in the activities obtained, as determined experimentally not only by the writer but also by other workers in this laboratory. The concentration of the buffer solution used was approximately 0.01 molar, but was non-toxic within a tenfold range of concentration.

Investigation

NATURE OF THE ACID CURVATURE

In order to gain a better understanding of the data, it was found necessary to determine first of all the nature of the acid curvature. This curvature is a phenomenon observed when split pea sections are placed in solutions of an acid pH. The sections show a strong backward curvature (fig. 1). VAN OVERBEEK and WENT (9) have shown that the growth curvature is due to the faster growth rate of the side with the intact epidermis in comparison with the cut surface. The acid curvature must therefore be due to the more rapid growth of the cut surface than of the intact surface. This would easily be explain-

[1] The citric acid used was of sufficient purity that growth curvatures as noted by VAN OVERBEEK and WENT (9) were not found.

able, on the basis of J. BONNER's (1) work, as being due to the ability of the buffer to penetrate from the cut surface. In the case of penetration of an acid buffer, a lowering of the internal pH on the cut surface would result, causing the formation of active non-dissociated auxin from the inactive dissociated auxin present.

There are two obvious means of testing this hypothesis: (1) measuring the growth in length of sections with and without epidermis immersed in buffer solutions of varying pH, and (2) determining the change in internal pH of sections with and without the epidermis when placed in solutions of varying pH. Data from such experi-

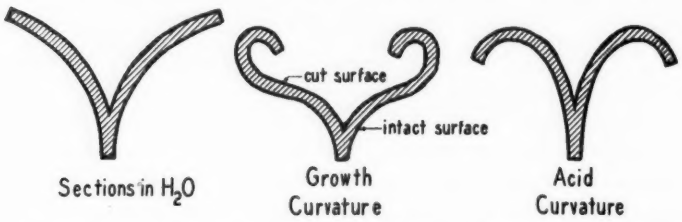


FIG. 1.—The pea test. Left: section of stem of etiolated pea seedling split longitudinally and placed in water. Center: similar section after treatment with solution containing an active auxin. Right: similar section after treatment with an acid solution containing no auxin.

ments are presented in tables 1 and 2. From these tables it is clear that: (1) Sections without the epidermis grow more rapidly at a pH of 4.0 than do intact sections at the same pH. At a pH of 7.0, however, the growth of the two is about the same. (2) Split sections when placed in a buffer solution of pH 4.0 show a change in internal pH corresponding to an increase of 2.7 times in amount of non-dissociated auxin present (from 3 to 8 per cent non-dissociation). The internal pH of intact sections on the other hand show no appreciable change.

The pH of the split sections was determined by splitting sections as for the regular pea test and floating them on the solutions for twenty-four hours. The split portions were then cut away from the solid base, ground in a mortar, the expressed sap decanted into the electrode vessel, and the pH determined with a glass electrode. The time between grinding of the sections and the initial pH determination was approximately one minute. Table 2 shows that this de-

crease in pH makes more auxin available for growth on the cut surface, giving rise to a growth gradient between the two surfaces, and therefore resulting in a backward curvature.

TABLE 1

GROWTH OF PEA SECTIONS WITH AND WITHOUT EPIDERMIS AT DIFFERENT EXTERNAL pHs

Sections	Time of measurement (hours)	Growth in length in mm.*	
		pH 7.0	pH 4.0
With epidermis	2	0.50	0.53
	20	0.78	0.90
Without epidermis	2	0.68	1.07
	20	0.60	1.06

* Values are averages of ten sections.

TABLE 2

RELATION OF INTERNAL TO EXTERNAL pH OF PEA SECTIONS WITH AND WITHOUT EPIDERMIS

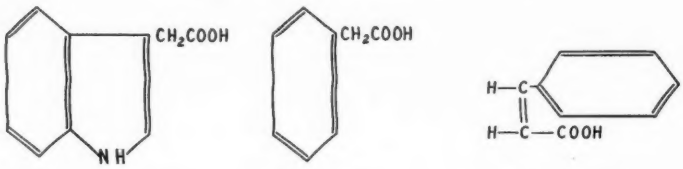
Type of section	No. of sections	External pH	Internal pH
Fresh intact plants	30	.....	6.25
Split part of sections in buffer solution eighteen hours	30	7.0	6.41
		6.0	6.18
		5.0	6.08
		4.0	5.70
Intact sections in buffer solution eighteen hours	30	8.0	6.19
		4.0	6.17

Evidently the acid curvature is caused by a higher active auxin concentration on the cut surface than on the surface with the intact epidermis.

RELATIONSHIP BETWEEN pK OF A GROWTH SUBSTANCE AND ITS ACTIVITY

The three substances used for the main body of this work are indole(3)acetic acid (pK = 4.75), phenylacetic acid (pK = 4.25),

and cis-cinnamic acid (pK = 3.85). The structures of these substances are shown in figure 2. The curves obtained for each of these substances by plotting degrees curvature in the pea test against the pH of the external solution are shown in figure 3, and the actual values for the curvatures are presented in table 3. These values



Indole (3) Acetic Acid | Phenyl Acetic Acid | Cis-cinnamic Acid

FIG. 2.—Three auxins active in the pea test

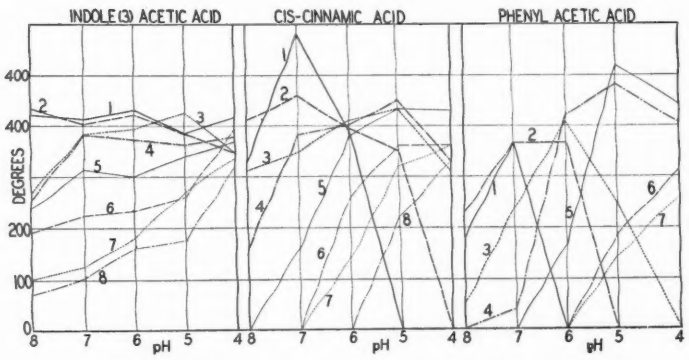


FIG. 3.—Effect of external pH on activity of auxins in the pea test. Curve numbers correspond to those in table 3.

represent the observed curvatures corrected for acid curvature. Obviously this must be done since at the more acid pH the observed curvature represents the resultant of two antagonistic reactions. At pH 7.0 there is only growth curvature, so that as the pH decreases these two reactions will tend to counteract each other, giving an apparent curvature much smaller than the true inward curvature.

Three points of interest can be noted in the curves presented in figure 3. (1) The slope of the curves obtained for phenylacetic acid and cis-cinnamic acid are much steeper than that for indole(3)acetic

TABLE 3

EFFECT OF EXTERNAL pH ON ACTIVITY OF AUXINS IN THE PEA TEST

Curve no.	Molar concentration	Pea curvature*				
		pH 8.0	pH 7.0	pH 6.0	pH 5.0†	pH 4.0†
1	Indole (3) acetic acid					
	2.0×10^{-4}	420	410	430	380	420
2	1.0×10^{-4}	430	400	420	380	350
3	5.0×10^{-5}	270	380	390	380	350
4	2.5×10^{-5}	260	380	360	360	380
5	1.2×10^{-5}	240	310	300	340	370
6	6.0×10^{-6}	190	220	230	260	390
7	3.0×10^{-6}	100	120	180	270	340
8	1.5×10^{-6}	70	100	160	150	320
1	Phenylacetic acid					
	32.0×10^{-4}	180	360	100	Toxic	Toxic
2	16.0×10^{-4}	230	360	370	0	Toxic
3	8.0×10^{-4}	50	240	410	250	0
4	4.0×10^{-4}	0	30	420	480	410
5	2.0×10^{-4}	0	0	170	450	450
6	1.0×10^{-4}	0	0	0	180	310
7	5.0×10^{-5}	0	0	0	140	260
8	2.5×10^{-5}	0	0	0	0	0
1	Cis-cinnamic acid					
	16.0×10^{-4}	330	580	380	Toxic	Toxic
2	8.0×10^{-4}	410	460	390	350	Toxic
3	4.0×10^{-4}	310	350	410	430	310
4	2.0×10^{-4}	160	380	360	450	330
5	1.0×10^{-4}	0	160	320	430	430
6	5.0×10^{-5}	0	0	260	360	360
7	2.5×10^{-5}	0	0	140	320	360
8	1.2×10^{-5}	0	0	0	220	330

* Each value averages six sections, or twelve separate curvatures.

† Observed values of curvature have 100° correction added for pH 5.0, and 210° for pH 4.0.

acid. (2) In the higher concentrations of phenylacetic acid and of cis-cinnamic acid extreme inhibition and toxicity occur at the lower pHs, while with indole(3)acetic acid slight inhibition occurs only at the highest concentration. (3) At a pH of 5.0 a concentration of

10^{-4} molal is just inhibitory for indole(3)acetic acid and for cis-cinnamic acid, while for phenylacetic acid a concentration of 2×10^{-4} molal is just inhibitory at pH 5.0. Each of these three points will be discussed later in the paper in the light of further considerations.

It was deemed advisable to determine whether a correlation exists between the pK of a growth substance and its activity in the pea test. This general type of correlation has been shown by J. BONNER (1) only in the *Avena*, and only for the natural auxin of the plant. Tests were made upon the two substances phenylacetic acid and cis-cinnamic acid, in concentrations that would give approximately the same reaction in the pea test, and from an external pH of 7.0 to 3.8 in intervals of 0.4 of a pH unit. The internal pH resulting from each external pH was determined and was used for correlative purposes. The pea curvatures were plotted against pH, and the dissociation curve was fitted to this. In figure 4 it may be seen that the correlation between the pK of a growth substance and its activity in the pea test is very good up to concentrations where inhibition sets in, for phenylacetic acid and for cis-cinnamic acid. This might well serve as an indication that these two substances take part in a manner similar to auxin in some pH dependent reaction.

As was shown by WENT (10), the curvature in the pea test is an exponential function of the concentration of the growth substance. By plotting the logarithm of the concentration against pea curvature, one obtains a straight line over a rather wide range of concentrations. For each pH, the logarithm concentration was plotted against curvature for the data presented in figure 3. It was seen that the curves obtained for each substance were similar, but that the curve for cis-cinnamic acid was shifted to the right (toward higher concentrations) of that obtained for indole(3)acetic acid, and the curve for phenylacetic acid was shifted even more so. This difference in activity between indole(3)acetic acid and cis-cinnamic acid was maximal at pH 8.0 and minimal at pH 4.0, showing that the pH effect is much more marked for cis-cinnamic acid than for the other acid. As mentioned earlier, J. BONNER, from work on the effect of acid on growth in the *Avena* coleoptile, postulated that auxin was active principally in the non-dissociated form. In order

that a substance with a pK less than that of auxin elicit the same growth response, a higher total concentration of this acid must be present in the cell in order that an equivalent amount of non-dissociated molecules be present. A correction for this difference in

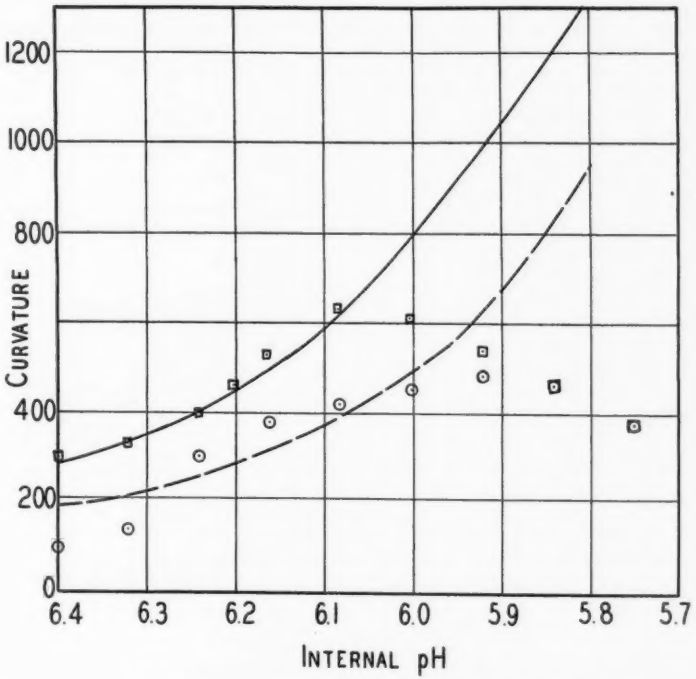


FIG. 4.—Correlation of internal pH, activity, and of pK:
——— dissociation curve of cis-cinnamic acid.
– – – – dissociation curve of phenylacetic acid.
⊡ pea curvature of cis-cinnamic acid.
⊙ pea curvature of phenylacetic acid.

degree of dissociation must thus be made before the activity of two substances with different dissociation constants can be compared. This can readily be done, taking for example two hypothetical substances *A* and *B*, *A* possessing the higher activity and the higher pK. To correct them to the same degree of dissociation at a given

pH, the observed activity of *B* must be multiplied by the ratio of the degree of dissociation of *A* to that of *B*, at the given pH. The degree of dissociation can be readily computed if the pK of the substances and the pH of the solution are known. This correction was made on the data presented in table 3, and the result is shown in figure 5. Figure 5 was computed at a pH of 6.2 (pH of the cell sap)

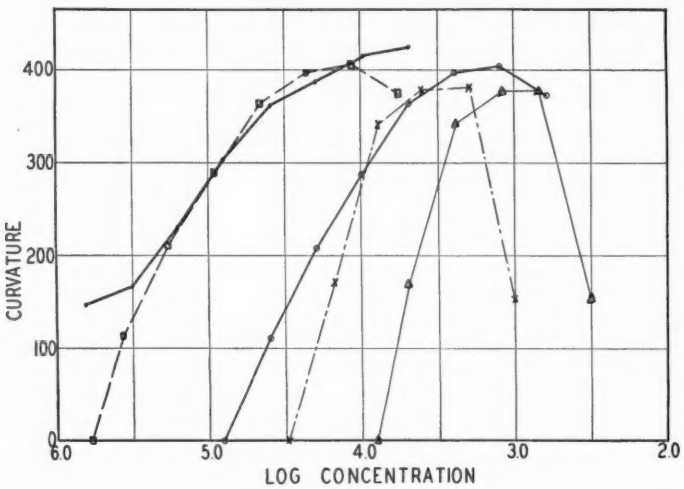


FIG. 5.—Activities of auxins corrected to the same percentage dissociation as that of indole(3)acetic acid at pH 6.2:

—— indole(3)acetic acid.

⊙——⊙ cis-cinnamic acid.

△——△ phenylacetic acid.

⊡---⊡ cis-cinnamic acid corrected to the same percentage dissociation as indole(3)acetic acid.

×---× phenylacetic acid corrected to the same percentage dissociation as indole(3)acetic acid.

by interpolation between pH 7.0 and 6.0. The experiment was repeated several times at pH 6.2, and the same result was always observed, as shown by figure 6. In figure 5 it is seen that over the major portion of the curve cis-cinnamic acid, when corrected for difference in degree of dissociation, has exactly the same activity as indole(3)acetic acid, differing only in falling off to zero activity at

higher concentrations than does indole(3)acetic acid and showing toxicity at the higher concentrations of free acid. Although the activity of phenylacetic acid was greatly enhanced by application of

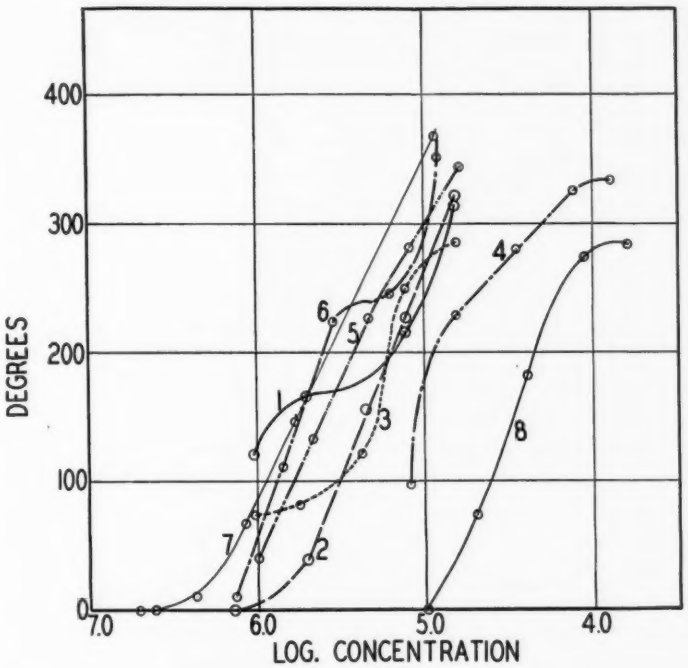


FIG. 6.—Activities of auxins corrected for differences in activities in pretreatment reaction, and for differences in dissociation constants.

1, indole(3)acetic acid.
2, indole(3)propionic acid.
3, indole(3)butyric acid.
4, indole(3)valeric acid.
5, napthaleneacetic acid.
6, anthraceneacetic acid.
7, cis-cinnamic acid.
8, phenylacetic acid.
pH, 6.2.

this correction, it was not brought to an activity equivalent to that of indole(3)acetic acid.

The explanation of the toxicity of cis-cinnamic acid is probably the following. In order that cis-cinnamic acid and indole(3)acetic

acid have present in the cell equal amounts of free acid required to elicit a given response, the total concentration of cis-cinnamic acid must be greater than that of indole(3)acetic acid since cis-cinnamic acid possesses the smaller pK. As mentioned earlier, both acids become toxic at the same total external concentration 10^{-4} molal, which might indicate that they enter the cell to the same extent. With the higher concentration of cis-cinnamic acid necessary to give the same growth response, the concentration of hydrogen ions in the cell must be greater with cis-cinnamic acid than with indole(3)acetic acid, thus changing the internal pH sufficiently to cause injury to the cells.

The observation that cis-cinnamic acid has the same molar activity as indole(3)acetic acid, if the physiological processes in which it must take part are limited in number (as in the pea test), and if corrections are made for differences in physical properties, leads to the view that under proper circumstances many substances might well possess equal activities upon cell elongation. WENT during the progress of this work made the interesting observation that two independent reactions are concerned in the pea test, (1) the growth reaction, and (2) a reaction strongly influenced by phenylbutyric acid, a substance showing no growth response whatever. This was demonstrated by pretreating pea sections with phenylbutyric acid, thereby greatly increasing their sensitivity to auxins. It seemed logical then that of the numerous compounds tested in the pea test many were not limited by reactiveness in the growth reaction, but by their reactiveness in the pretreatment reaction. Several compounds were tested, therefore, using sections pretreated for two hours with phenylbutyric acid at a concentration of 100 mg./liter, and buffered at pH 6.2. Buffering was used merely to prevent excessive acid curvatures. Using this method, and correcting for differences in pK when possible, the following compounds were found to possess the same activity at pH 6.2 as indole(3)acetic acid (fig. 6). Indole(3)propionic acid, indole(3)butyric acid, napthaleneacetic acid, anthraceneacetic acid, and cis-cinnamic acid. The activities of indole(3)valeric acid and of phenylacetic acid, although enhanced over previously reported values (**3, 6**), were not brought to the activity of indole(3)acetic acid. By using this pretreatment with phe-

nylbutyric acid, cis-cinnamic acid no longer dropped to zero activity before indole(3)acetic acid, showing that at lower concentrations the activity of cis-cinnamic acid in accessory processes had become the limiting factor. It is now possible to advance a hypothesis for the difference in the slopes of the curves shown in figure 3, where degrees curvature in the pea test were plotted against pH for the three different growth substances. There is definitely more than one process concerned in the pea test, and these probably are not all affected by pH. In fact it has been shown that the process influenced by pretreatment with phenylbutyric acid is independent of pH. For substances having equal activities in a pH dependent reaction but different activities in a pH independent, their behavior with change of pH will be a function of their relative activities in each of the two processes. The substance with the least overall sensitivity to pH will then have the smallest slope. The activity of indole(3)acetic acid is not accurately determined in the pretreatment reaction, so at present little can be said as to the relative activities of indole(3)acetic acid, phenylacetic acid, and cis-cinnamic acid in this reaction. In case it actually possesses a low activity in the pretreatment reaction, this would give an explanation for the difference in slopes noted in figure 3.

Discussion

The properties that a growth hormone of the auxin group must possess might be broadly classified into two general groups: ability to take part in a stoichiometric reaction which results in cell elongation, and ability to furnish the necessary "environment" for this reaction (1). For example, it must be able to take part in the "pretreatment reaction." In addition to these two properties there is the third property of transportability discussed by THIMANN (8). Any one of these three factors may, depending upon the method of assay, limit the apparent activity of a substance, and thereby obscure its activity in the primary reaction (1) already mentioned. Thus THIMANN showed that the activity of a substance was limited in the *Avena* test by its transportability, so that by the use of the pea test where this plays no part many more compounds were shown to possess growth activity, although to varying extents. For example,

cis-cinnamic acid was found to have 10 per cent (7)[2] of the activity of indole(3)acetic acid. It has been shown in the present paper that if corrections for the differences in pKs are made so that equimolar concentrations of free acid are compared, cis-cinnamic acid has an activity per molecule equal to that of indole(3)acetic acid. Still another reaction may limit the activity of a substance in the pea test. This reaction is a preparatory reaction, and as mentioned above can be carried out by substances having no true growth activity; for example, by phenylbutyric acid. If this reaction is made non-limiting by pretreatment with phenylbutyric acid a still closer approach may be made toward measuring the primary growth activity of a substance. In this way several more compounds were shown to possess the same activity per molecule as indole(3)acetic acid. It may be concluded from this that the difference in activities observed for different auxins in the pea test (3, 7) is due to their activities in various accessory reactions, but that in the final growth reaction, pH dependent and stoichiometric in nature, they all possess the same activity.

From the work of HAAGEN SMIT and WENT (3), KOEPFLI, THIMANN, and WENT (7), and of KÖGL and KOSTERMANS (6), an essential molecular structure for a growth promoting substance was formulated. The requirements are (1) a carboxyl group, (2) a ring structure, (3) one double bond in the ring structure, (4) a minimum distance of at least one carbon atom between the carboxyl group and the ring, and (5) a definite steric configuration. Inasmuch as no compounds lacking in these requirements have been found to give the growth reaction, it would seem that these are also the requirements for the final reaction. Thus one might conclude on the basis of the experiments reported here that all compounds possessing these basic structural requirements will be equally active in the reaction resulting in cell elongation, and any difference in activity will be due to varying activities in accessory reactions.

[2] HAAGEN SMIT and WENT (3) reported cis-cinnamic acid as having the same activity as indole(3)acetic acid in the pea test. They compared at pH 7.0 concentrations that gave high pea curvatures, and so were working at high concentrations where the pK factor is less important as may be seen from figure 5.

Summary

1. It is concluded that the acid curvature in the pea test is due to a greater growth of the cut surface than of the intact surface. This is explained as due to a higher concentration of active auxin on the cut surface, caused by a measurable decrease of the internal pH of the cut surface, when split pea sections are placed in acid buffers.

2. A correlation is found between the dissociation curves and the activities at different internal pHs of cis-cinnamic acid and phenylacetic acid.

3. By correcting for difference in pK, so that only equimolar concentrations of the free acid are compared, it is found that cis-cinnamic acid possesses the same activity as indole(3)acetic acid in the pea test. The activity of phenylacetic acid, although enhanced, was not so high as that of indole(3)acetic acid.

4. By pretreating pea sections with phenylbutyric acid and correcting for difference in pK when possible, the following compounds are found to possess molar activities equal to that of indole(3)acetic acid in the pea test: indole(3)propionic acid, indole(3)butyric acid, napthaleneacetic acid, anthraceneacetic acid, and cis-cinnamic acid. The activities of phenylacetic acid and of indole(3)valeric acid, although increased, are not brought to that of indole(3)acetic acid.

5. It is suggested that compounds possessing the essential molecular structure probably all have the same activity in the pH dependent, and stoichiometric growth reaction, and that the differences in their observed activities are due to differences in activities in secondary processes.

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ORIGIN AND DEVELOPMENT OF SHOOTS FROM THE TIPS OF ROOTS OF POGONIA OPHIOGLOSSOIDES

MARGERY C. CARLSON

(WITH THIRTEEN FIGURES)

Introduction

While observing plants of *Pogonia ophioglossoides* (L.) Ker. in a sphagnum bog in northern Wisconsin, it was noticed that the new shoots arose from, or near, the tips of the roots. Adventitious buds are of common occurrence on roots, many plants being propagated by root cuttings; but only a few cases of shoot formation similar to that in *Pogonia* have been recorded.

BEIJERINCK (1) distinguished between those adventitious buds which originate from the phellogen, or from the wound callus, of root cuttings and those formed normally, or after injury, from the primary tissues of the root, but studied only the latter. He described briefly the formation of buds from the outer layers of the cortex of the roots in *Aristolochia clematitis*, *Linaria vulgarus*, and *Orobanche galii* and other parasites. TRÉCUL (10) reported the endogenous production of shoots from root cuttings of *Maclura aurantiaca*, *Tecoma radicans*, *Ailanthus glandulosa*, and *Paulownia imperialis*. WARMING (13) found buds arising in the outer cortical layers in certain members of the Podostemaceae. PRIESTLEY and SWINGLE (7) described in detail the origin and development of adventitious buds in root cuttings of *Crambe maritima*, in which certain cells of the phellogen near a wound become more actively meristematic than their neighbors and produce a group of dividing cells which soon differentiate into the growing point of a bud. GRAHAM and STEWART (3) described stages in the development of buds from the cambium of root cuttings of *Anchusa italica*.

The few cases where the tip of the root becomes transformed into a shoot have been summed up and described by ROSTOWZEW (9). In *Neottia nidus-avis*, PRILLIEUX (8), WARMING (12), IRMISCH (5),

and others found that a swelling appears, either on the end of the root or laterally just back from the tip, and differentiates into a bud with alternating, semicircular leaf primordia and with a cluster of roots arising from the base of the bud as it elongates into a shoot. If the bud is lateral, the tip of the main root continues to grow forward. It was suggested that these adventitious "root buds," with their roots, may provide a method of vegetative propagation for this plant; but BEIJERINCK and IRMISCH state that they are only occasionally produced in nature, and that when produced they often die prematurely. BEIJERINCK noted that adventitious roots were produced exogenously in great numbers from the rhizomes of *Neottia*, and suggested that there might be a close anatomical relationship between the stem and the root in this plant, as in *Corallorhiza* and *Epipogium*. GOEBEL (2) found on three plants of *Anthurium longifolium* that terminal "root sprouts" were produced in a manner very similar to that in *Neottia*. KARSTEN (6) reported the production of flowers from a swelling on the end of the root of a plant of *Dioscorea* in a greenhouse in the hot summer of 1858. SACHS claimed that the tip of the root of *Platycerium willinkii* becomes transformed into a bud, and ROSTOWZEW (9) described fully this transformation in the preceding and in other species of *Platycerium* and in *Asplenium esculentum*. VAN TIEGHEM (11) and HOLLE (4) described the formation of a shoot from the tip of the root in *Ophioglossum vulgatum*. They reported that a root develops endogenously from the adventitious shoot and grows in the direction of the mother root, thereby making the shoot appear to arise laterally from the main root, as a sort of "embryo" formed from the root meristem.

A peculiar situation appears in *Rumex acetosella*, which according to BEIJERINCK (1) ordinarily produces buds endogenously in a ring about the base of a secondary root, when it grows horizontally close to the surface of the ground; but which on one occasion produced a bud on a root cutting, the bud then becoming transformed into a root. His figures show a branch root with root cap and root hairs, having a ring of reduced leaves at its base, and he suggested that the bud began to develop, then suddenly changed its growing point into that of a root tip. Other investigators express doubt as to the accuracy of these observations.

Investigation

This study was made from hand sections of fresh material and from stained serial sections of material imbedded in paraffin.

The roots of *P. ophioglossoides* grow horizontally in the sphagnum mat just above the level of the standing water in the bog. They are 1 to 1.5 mm. in diameter throughout their length, are unbranched,

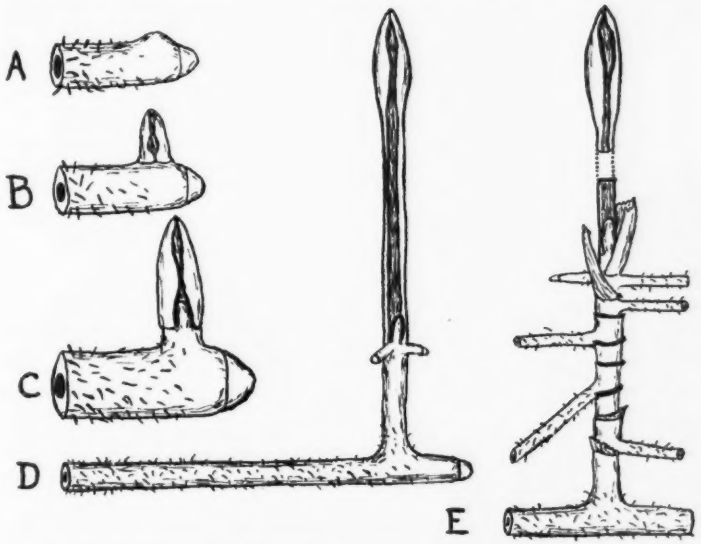
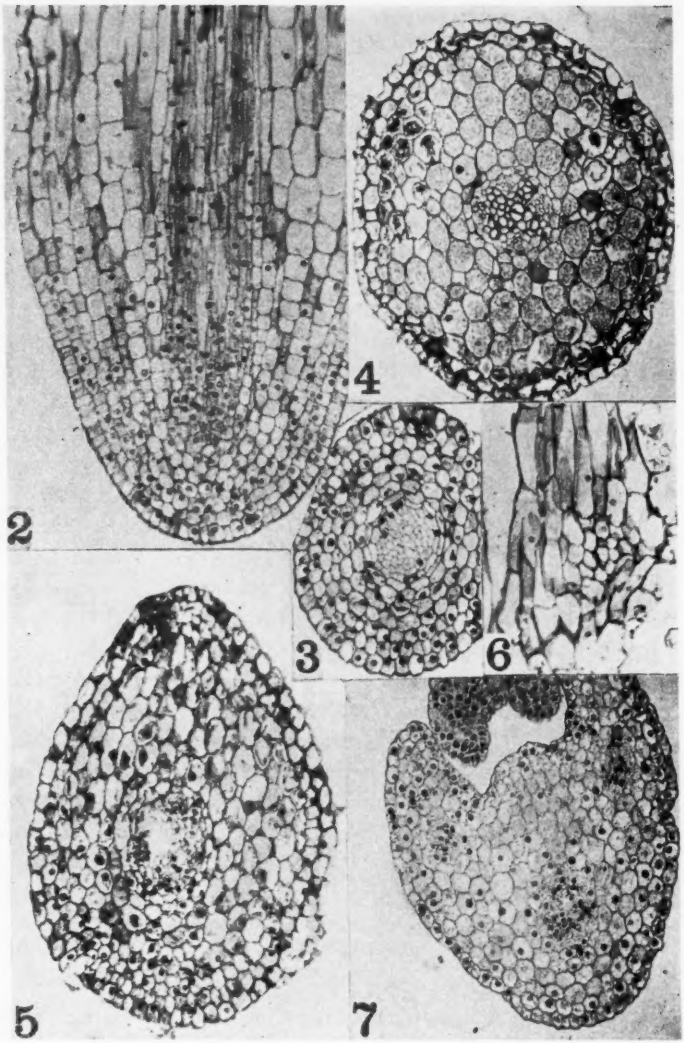


FIG. 1.—*A:* portion of end of root showing protuberance on upper side. *B:* same, protuberance differentiated into young shoot. *C:* older shoot showing elongated outer leaf, primordium of second leaf, and slight elongation of axis below first node. *D:* still older shoot showing differentiation of outer leaf into petiole and blade, second leaf further developed, and two adventitious roots arising from first node. *E:* much older shoot showing nine nodes, each with leaf or leaf scar and sometimes with one or two adventitious roots; tip of main root grown forward; root hairs persisting.

coarsely hairy, and are usually green. The short tapering tips are smooth. A longitudinal section of the tip of the root (fig. 2) shows its unusual structure. No root cap is present. The promeristem is shorter and the cells are more vacuolate, their cytoplasm less dense, and their nuclei smaller in proportion to the size of the cells than is typical for such regions in roots. Differentiation of the histogens be-



FIGS. 2–7.—Fig. 2, longitudinal section of tip of root showing early differentiation of tissues; no root cap present. Fig. 3, cross section of root through region of primary meristems showing beginnings of tissue differentiation. Fig. 4, same through mature region showing tetrarch xylem and phloem; mycorrhizal fungus in some and starch grains in other cortical parenchyma cells; thick walls between epidermis and hypodermis. Fig. 5, cross section of root showing cell enlargement on one side; at peak of swelling the cells have divided; an early stage in shoot formation. Fig. 6, tangential section of root showing group of dividing cells in cross section. Fig. 7, cross section showing two leaf primordia of developing bud, outer one showing three provascular strands; inner one showing no differentiation of tissues.

gins early. A well defined layer of epidermal cells, with their outer walls conspicuously thickened, completely covers the tip of the root, and the provascular strand becomes evident about 0.1 mm. back from the tip. Lateral enlargement and elongation of cells can be followed in figure 2. Distally from the place where lateral enlargement of cells reaches its maximum, the outer walls of the epidermal cells are thinner than below, and are frequently broken on the more mature parts of the root. The inner walls of these cells, however, are distinctly thicker here than in the meristematic region.

A cross section of the root at a level where the primary tissues are beginning to differentiate is shown in figure 3. Its protostelic nature is evident. The matured region (fig. 4) shows the radial and tetrarch arrangement of the primary xylem and phloem. The protoxylem consists of spiral and scalariform tracheids; the metaxylem consists of vessels, tracheids, and a few parenchymatous cells. The phloem, consisting of sieve tubes and parenchymatous cells, lies between the radiating strands of xylem. All the cells of the provascular strand mature into xylem and phloem, so that no cambium remains. The steles vary from triarch to hexarch, the tetrarch condition being most common. The pericycle and endodermis are single layered parenchymatous tissues. The Casparian strips in the endodermal cells are conspicuous. The cortex consists of from five to seven layers of loosely arranged parenchymatous cells, usually filled with starch grains. The outermost cortical layer (hypodermis) is more like the epidermis than the rest of the cortex: the cells are tightly fitted together and contain no starch grains. The walls between the hypodermis and the epidermis are much thicker than the outer walls of the epidermis.

Root hairs are produced in the usual manner from a few of the epidermal cells. Their walls are somewhat thickened, however, and they persist all along the root as a sparse, coarse fuzz.

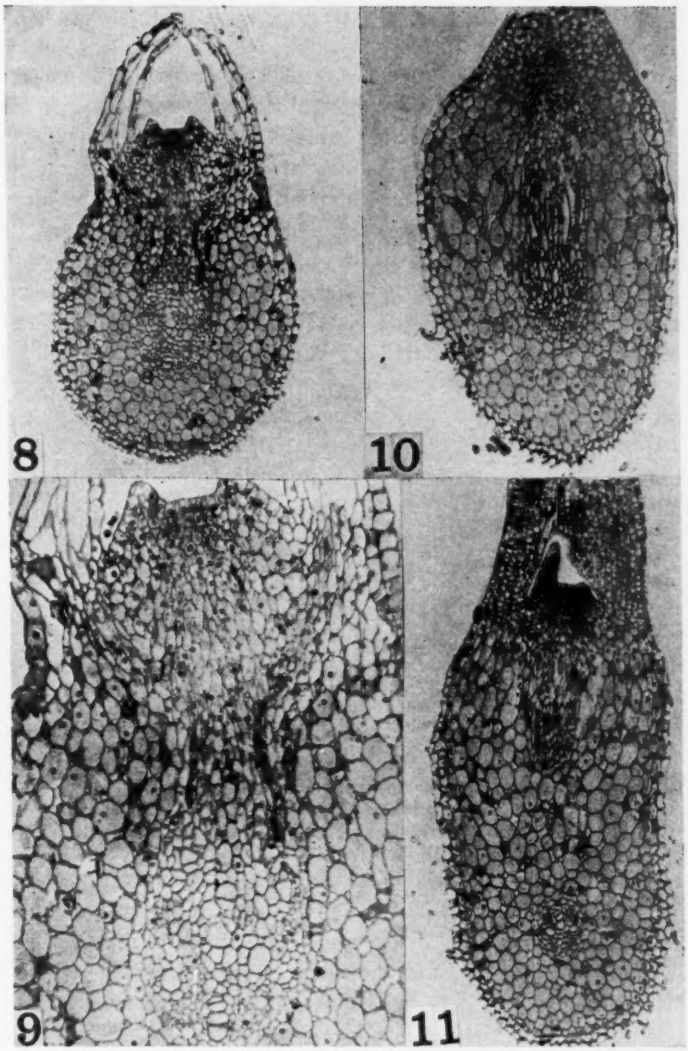
A mycorrhizal fungus enters the root hairs or external walls of other epidermal cells, passes through the hypodermis and often through one or two adjacent layers of cells, and forms a tangled mass of hyphae in the cells in the next several layers of the cortex (fig. 4). Upon entrance of the fungus into a cell, the reserve starch begins to disappear, the nucleus at first enlarges, and then becomes

indistinguishable in the mat of fungal hyphae. The mycelium seems never to penetrate deeper than the inner cortical cells.

The adventitious shoot begins its development as a protuberance on the side or on the end of a root (fig. 1*A*). The epidermal and cortical cells enlarge, chiefly in the radial dimension and form a swelling, at the peak of which the cells begin to divide. Since the daughter cells do not immediately enlarge, a group of small cells, destined to become a bud, is formed (figs. 5, 6). The protuberance enlarges as the cells continue to divide and form the promeristem of a stem tip (figs. 1*B*, *C*; 8), the outer part of which differentiates into a dome-shaped leaf (fig. 11). A second leaf begins as a semicircular ridgelike primordium arising from the promeristem (figs. 7, 9).

Elongation of the shoot begins with the radial and tangential enlargement of the epidermal and cortical cells of the root between the stele and the stem tip, early stages of which are seen in figures 1*C* and 8, and successively later stages in figures 10 and 11. The cells of the lower side of the root have not changed at all. The outer sheathing leaf elongates (figs. 8, 11), the inner leaf primordium enlarges, and later leaf primordia form successively within one another.

A study of a series of cross sections of the shoot from the tip downward shows the differentiation of tissues and the transition between the tissues of the shoot and root. Three or more small provascular strands appear just below the promeristem in a ring surrounding a small pith region. At a lower level, but before much maturation of cells has occurred, three strands from the outer leaf extend through the cortex and join the ring of bundles. Farther down, where the cells of the stem are mature (fig. 12), there is an epidermis, the outer walls of whose cells are somewhat thickened; the cortical parenchyma, with starch grains and mycorrhizal fungus; the endodermis, with Casparian strips; the parenchymatous pericycle; the ring of three vascular bundles; three leaf traces in the cortex; and the small three pointed pith. The xylem of a bundle partially incloses a small strand of phloem, which lies toward the outside. The xylem of adjacent bundles is in contact. Neither the xylem nor the phloem is highly specialized. No cambium is present. The cells of the pith may become thick walled. The vascular tissues continue as described down to the stele of the root, where they



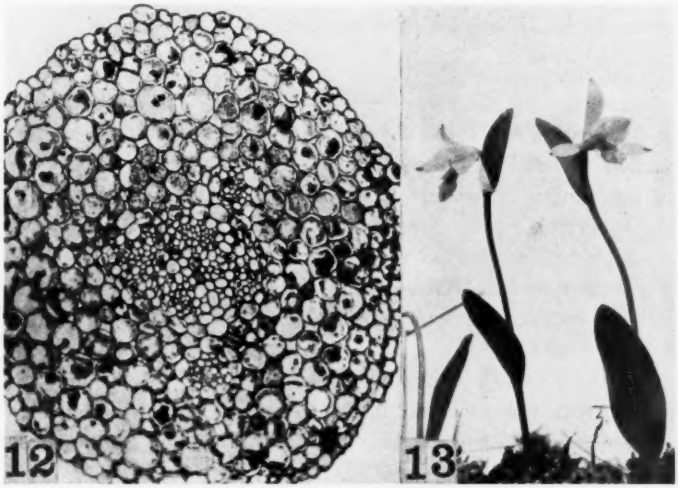
FIGS. 8–11.—Fig. 8, bud showing first two leaf primordia in longitudinal section on side of root; vascular tissues between bud and stele of root beginning to differentiate. Fig. 9, portion of bud shown in fig. 8 enlarged. Fig. 10, bud (not median section) showing early elongation by enlargement of cells between stele and bud; further differentiation of vascular tissues between root and bud; undifferentiated median trace of first leaf merging with transition vascular tissues. Fig. 11, later stage of growing shoot; first leaf elongating, especially at base; primordium of second leaf further developed than in fig. 8; greater elongation in region between root and first node.

merge with those of the root. Here the stele of the root is enlarged on the upper side by the production of much xylem (figs. 8, 9). The characteristic arrangement of xylem and phloem is evident, but the upper radial plate of xylem is much larger than the others, and the two strands of phloem on either side of it are displaced laterally by the added xylem tissue. The endodermis and pericycle are not differentiated about the stele on the side toward the developing bud, but the cells between the vascular tissues of the shoot and root mature so that a connection is established. It must be remembered that the differentiation of tissues occurs simultaneously in shoot and root; hence the connection of their xylem, phloem, pericycle, and endodermis is present from the beginning of differentiation. The pith of the shoot ends at the stele of the root. Epidermal hairs similar to those on the root are often present on the basal part of the stem.

Further growth and differentiation of root and shoot proceed normally. The part of the shoot below the first leaf may elongate so that the first node is 0.1 to 4.0 cm. above the root. The sheathing petiole of the first leaf elongates, pushing above the surface of the surrounding sphagnum. The upper end broadens and unfolds into a narrowly oval blade which is usually about 3–6 cm. long and 0.5–1.0 cm. wide when mature. The stem tip develops very slowly, and the second leaf, which lies in a groove of the petiole of the first leaf, gradually elongates, differentiates, and is pushed upward by elongation of the first internode.

While the first leaf is developing, one or more adventitious roots are usually produced at the first node (fig. 1*D*), just above or below the insertion of the leaf. The early stages in their development are very similar to those in the development of the adventitious buds. They are exogenous; that is, they originate from the epidermal and cortical cells of the stem, which at a certain point near the leaf insertion begin to divide, forming a group of meristematic cells which appears as a swelling on the surface. This group of cells becomes organized into a root tip which grows and extends outward at right angles to the stem. If the root is formed above the attachment of the leaf, it pushes through the petiole. Vascular tissues are differentiated in the developing root and in the cortex of the stem

connecting the new root with the stele of the stem. These adventitious roots grow horizontally, in a plane somewhat above that of the first root, and are similar to it in appearance, structure, and behavior. One leaf is produced at each node (fig. 1*E*), the lower ones dying and leaving only shriveled bases or scars. One or two green and coarsely hairy adventitious roots may be produced on each



FIGS. 12, 13.—Fig. 12, cross section just below first node of stem showing epidermis, cortical parenchyma with starch grains and mycorrhizal fungus, endodermis, pericycle, three vascular bundles, three leaf traces (from first leaf) in cortex, and small three angled pith region. Fig. 13, flowering shoots showing sessile leaves on aerial stem and bracts subtending the flowers.

node. The root, or roots, at the first (lowermost) node are usually the first to show evidence of shoot formation near their tips.

The seasonal history of the growth of the shoots has not been worked out carefully, but one leaf seems to appear above the sphagnum mat each season, each successive one taller and larger than the previous one, until finally the terminal bud produces an aerial flowering stem, 20 to 35 cm. high, with two or three leaves: a long stalked leaf near its base, one without a petiole midway up; and a leaflike bract subtending the flower (fig. 13). The number of

years elapsing between the first appearance of a bud and the production of a flowering stalk from this bud is not known.

Each new series of roots, growing horizontally from the nodes of the shoots, occupies a plane in the sphagnum mat a little higher than that of the previous series, and the new shoots originating from the ends of these roots are therefore also higher. The distance apart of these successive layers of roots may bear a relationship to the rate of growth of the sphagnum, but no measurements are available to verify this point.

Summary

1. The roots of *Pogonia ophioglossoides* are horizontal, unbranched, covered with persistent root hairs, and green, at least at their extremities. They are protostelic, radial, triarch to hexarch, with endodermis having conspicuous Casparian strips, and root hairs. They have no root cap.

2. An adventitious shoot begins as a swelling on the root, either at its end or on the upper side a short distance back from the tip. Lateral shoots on roots are more common than terminal ones. The swelling is formed by enlargement and division of the cells of the epidermis and cortex of the root, usually on the upper side, producing a group of meristematic cells which differentiates into a typical bud.

3. The new shoot produces one leaf at each node, and probably one leaf each season, until the shoot is old enough to flower.

4. If the adventitious shoot is lateral, the root may grow forward and produce another shoot the following season. One or two adventitious roots are produced exogenously at most nodes of the adventitious shoot.

5. The formation of exogenous adventitious shoots from tips of roots and of exogenous adventitious roots from shoots is the normal habit of growth in this species of *Pogonia*, and is apparently of comparatively rare occurrence among plants.

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VITAMIN B_1 AND THE GROWTH OF GREEN PLANTS[1]

JAMES BONNER AND JESSE GREENE

(WITH TWO FIGURES)

Introduction

Vitamin B_1 is of importance as a growth factor for the roots of higher plants. Pea roots will grow in vitro in an otherwise optimal nutrient solution only if an adequate supply of vitamin B_1 is present (1, 3), and the same is true for excised roots of tomato (12). Vitamin B_1 should be regarded both as a "vitamin" for the growth of roots in vitro and certainly as a phytohormone in the green plant. Earlier work in animal nutrition has shown that vitamin B_1 is present in the leaves (2). It is formed there only in the presence of light (9). It would seem logical to suspect that the root receives its supply of vitamin B_1, either directly from green leaves, or indirectly from the seed, where vitamin B_1 is stored (2).

When plants are grown under optimal external environmental conditions such that no external factor can be said to limit growth, it is nevertheless found that the growth rate of the plant is limited. It is clear that under optimal external conditions, certain internal factors must determine the plant's growth. The ability to synthesize vitamin B_1 would be one such internal factor. A plant supplied with optimal light intensity, temperature, amounts of nutrients, etc., might still fail to supply to its roots an amount of vitamin B_1 sufficient to enable the root to fulfil its full potential growth. This would then be a hypothetical specific case of limitation by an internal factor.

The results recorded in the present paper, while of a preliminary nature, nevertheless indicate that because of its function as a root growth factor, vitamin B_1 may be of some importance in practical agriculture.

[1] Report of work carried out with the aid of the Works Progress Administration, Official Project Number 465–03–3–342, Work Project Number N-9199.

Investigation

Vitamin B_1 as a phytohormone

The following experiment was designed to show directly that the root is dependent upon the green leaf for its supply of vitamin B_1. Pea seeds were germinated upon filter paper in a physiological darkroom. When the epicotyl had attained a length of approximately 4 cm., the seedlings were divided into two lots of 100 each, and the cotyledons were removed from all the plants. One lot of 100 seedlings was then planted in sand in the greenhouse and supplied daily

TABLE 1

Relation of vitamin B_1 content of leaf and root tip of pea plants with cotyledons removed

Plant environment	Plant part	Vitamin B_1 content: mg. $\times 10^6$			
		Days after removal of cotyledons			
		0	4	6	8
In dark	1 cm. root tip.....	15	15	15	18
	Apical bud and two apical leaves.....	28	32	32	32
In light	1 cm. root tip.....	15	16	34	46
	Apical bud and two apical leaves.....	28	36	46	100

with nutrient solution (**10**). The other lot was kept in the darkroom and supplied nutrient solution containing sucrose. At the time of removal of the cotyledons, and after four, six, and eight days, root tips 1 cm. long were removed and assayed for vitamin B_1 by the *Phycomyces* test (**6, 14**). At the same time the apical buds and two apical leaves were assayed in the same way. The results of one representative experiment are shown in table 1.

The tips of the primary root as well as the apical buds of the primary axis (at the time of removal of cotyledons) were found to contain a relatively large amount of vitamin B_1. In the plants which remained in the dark, the vitamin content of both root tip and bud

with the apical leaves remained approximately constant. In the series in the light the vitamin B_1 content of the bud and young apical leaves increased rapidly. Over the same period of time the amount present in the root tip also increased. The higher vitamin B_1 content of the roots in the light series is correlated with its synthesis in the apical portion of the plant, and indicates that it is supplied to the roots from the green leaves.

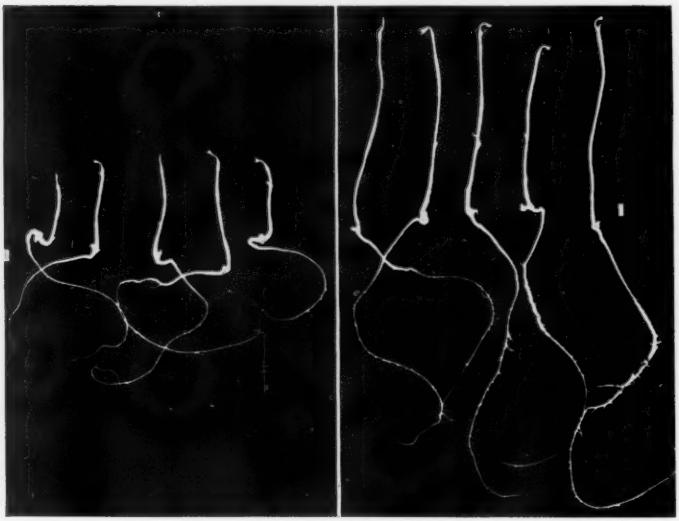

FIG. 1.—Pea embryos from which cotyledons were removed and then grown in dark under sterile conditions on nutrient medium of known composition. Left, without added vitamin B_1; right, with added vitamin B_1 (0.1 mg. per liter). Small rectangle indicates approximate size of initial embryo. Photographed after four weeks.

RELATION BETWEEN ROOT GROWTH AND SHOOT GROWTH

When excised pea embryos (without cotyledons) are grown in vitro in the dark upon a nutrient medium containing sucrose, nitrate, and other essential inorganic salts, the growth of the shoot is increased if small amounts of vitamin B_1 are added to the medium (4, 11), as shown in figure 1. Excised buds, however, grown in vitro upon the same medium, do not respond to added vitamin B_1 with increased growth. It seems probable, therefore, that whatever effect

vitamin B_1 exerts in increasing the growth of the shoot is due primarily to the effect of this substance upon root growth. WENT (18) has shown that shoot growth of etiolated pea seedlings from which the roots were removed is greatly decreased, even under conditions which assure an adequate food and water supply. According to WENT, some specific substance necessary for the growth of the shoot is formed in the active root, and when roots are cut off the locus of formation of this specific substance is therefore removed. There are of course other ways in which root activity might benefit shoot growth, in the case of the normal green plant.

EXPERIMENTS WITH GREEN PLANTS

Plants were grown in the greenhouse in washed sand in two gallon glazed crocks, provided with drainage. Two to several plants were grown in each crock, the number depending upon the type of plant. Selection carried out as carefully as possible assured that the control (without B_1) and the experimental (with B_1) plants were closely comparable. HOAGLAND'S (10) nutrient solution was used. This was supplied at the rate of 500 cc. per pot every two days and the pots flushed with tap water on the intervening days, frequent flushing being adopted in order to decrease as far as possible complications owing to the presence of microorganisms.

Vitamin B_1 was added to the nutrient solution in concentrations of 1, 0.1, and 0.01 mg. per liter.[2]

Pea (variety Perfection), bean (Kentucky Wonder), radish (Scarlet Globe), and tomato (Bonny Best) failed to show any significant response to vitamin B_1, when added to the nutrient solution in the concentration of 1 mg. per liter. It is realized that the cultural conditions of the present experiments were not ideal and the growth of these rapidly growing varieties may have been limited by nutrient supply rather than by internal factors. The fact that these plants grow rapidly and produce luxuriant root systems, however, indicates that they synthesize their internal growth factors (such as vitamin B_1) in amounts sufficient for ordinary growing conditions. In one experiment, beans grown in tank cultures with no limitation

[2] The writers are indebted to Merck & Co. for the supply of synthetic vitamin B_1 hydrochloride.

of mineral nutrients showed a considerable increase in rate of shoot growth in response to vitamin B_1 (0.1 mg. per liter).

Several varieties of plants noted as being slow growing were subjected to similar experimentation. The results are summarized in table 2. The tung oil tree (*Aleurites fordii*) is an example. Rooted cuttings (20) which had been potted for several months and whose growth had entirely ceased were used. The control plants grew well but the plants receiving vitamin B_1 (1 mg./liter of nutrient) showed

TABLE 2

EFFECT OF VITAMIN B_1 UPON SHOOT GROWTH OF PLANTS GROWN IN SAND CULTURE

Species	Number of plants	B_1 concentration (mg./l.)	Duration of experiment (weeks)	Average shoot growth, cm./plant for total period		
				Control	Vitamin B_1	Vitamin B_1 / Control
Aleurites fordii*	6	1	10	7	13	1.86
Buginvillea glabra†	6	1	10	10.7	14.0	1.3
Arbutus unedo†	8	1	10	10.1	13.3	1.3
Eucalyptus ficifolia†	6	1	9	11.1	16.3	1.5
Camellia japonica‡	24	0.1	5	0.0	2.0	.....
Camellia japonica‡	.....	0.01	5	0.0	2.4	.....

* Rooted cuttings.

† Selected seedlings kindly supplied by Dr. W. Lammerts, Armstrong Nurseries, Ontario, Calif.

‡ Seedling plants kindly supplied by Mr. J. Youtz.

almost twice as much total shoot elongation after two and one-half months. Upon removal of the plants from the crocks at this time, it was found that the root systems of the vitamin B_1 treated plants were more luxuriant than those of the control plants (fig. 2). The response to vitamin B_1 was similar to that shown by etiolated pea embryos; root growth was improved, and correlated with this was considerable increase of shoot growth.

With the other plants listed in table 2, the effects of added vitamin B_1 are in no case great but are nevertheless definite, despite the small numbers of plants used. *Hardenbergia comptoniana* actually grew considerably less when vitamin B_1 was supplied to it. In view of the results with lower concentrations, as detailed later, it is appar-

FIG. 2.—Tung oil trees grown in washed sand with nutrient solution. Plants on right received no vitamin B_1; plants on left received vitamin B_1 (1 mg. per liter). See table 3.

ent that a concentration of 1 mg. per liter is much higher than necessary, and might in some cases prove toxic.

Twenty-four selected *Camellia* plants approximately 17.5 cm. tall were divided into three lots and were then supplied respectively with no vitamin B_1 or with concentrations of it at 0.1 or 0.01 mg. per liter. All the plants were in the temporary resting state. During the five weeks of the experiment none of the control plants resumed growth, whereas seven of those treated with 0.01 mg./liter became active and grew in length an average of 2.4 cm. per plant.

TABLE 3

EFFECT OF VITAMIN B_1 ON SHOOT GROWTH OF PLANTS GROWN IN SOIL

Species	Total plants	Duration of experiment (weeks)	B_1 concentration (mg./l.)	Average shoot growth, cm./plant		
				Control	Vitamin B_1	Vitamin B_1 / Control
Arbutus unedo.....	7	8	1	2.7	6.1	2.3
Prunus ilicifolia.....	11	8	1	4.0	6.4	1.6
Bryophyllum sp.....	50	5	1	0.72	1.78	2.5

Other varieties showed increased shoot growth when vitamin B_1 was added to the soil every other day in the water given to the plant (table 3). *Arbutus unedo* and *Prunus ilicifolia* were grown outdoors in pots under conditions approximating ordinary nursery treatment. A comparison of tables 2 and 3 shows that *Arbutus* grows much more slowly under these conditions than in more favorable greenhouse sand culture environment, but nevertheless responds vigorously with increased shoot elongation when vitamin B_1 is added.

Bryophyllum daigremontiana and *B. tubiflora*, grown in the greenhouse in light potting soil, responded to added vitamin B_1 with considerable increases in rate of shoot and leaf development. Although the experiment recorded in table 3 was done with a 1 mg./liter solution of vitamin B_1, a second experiment with 0.01 mg./liter also showed a beneficial effect. The lowest limit of effective concentrations has not yet been determined.

Organic fertilizers and other sources of vitamin B_1 in the soil

Although a wide variety of organic substances are to be found in soil (**15, 16**), yet their entire significance or importance has remained uncertain. In practice, organic fertilizers such as stable manure are often of distinctly more benefit to plant development than is a purely inorganic fertilizer. Russell (**13**) states, "There is no doubt that the plant can grow satisfactorily and attain full development with inorganic nutrients only. But numerous field observations suggest that in the soil some of the organic matter may

TABLE 4

Vitamin B_1 content of manure and other materials

Material	Vitamin B_1 content*
Arizona steer manure†	0.13 mg./kilo
Local steer manure	0.08
Dairy manure	0.13
Alfalfa plant	5.0
Azotobacter‡	140

* All figures based upon air-dry weight of sample.

† Manure samples through courtesy of Dr. A. D. Shamel, U.S. Dept. Agr., Riverside, California.

‡ Sample of Azotobacter grown upon synthetic medium, through courtesy of Dr. G. Hilbert, U.S. Bureau of Chemistry and Soils, Washington, D.C.

play a direct part. No combination of artificial fertilizers is as effective as farm yard manure in maintaining a high uniform level of crop production from year to year." With no desire to over simplify the problem of the effectiveness of such manures, the writers desire to point out one specific manner in which manure, aside from its content of the usual nutrients, may benefit the plant. As shown in table 4, manure of different varieties contains appreciable amounts of vitamin B_1, as determined by the *Phycomyces* test (**6, 14**).[3]

The samples tested contained approximately 5 per cent (or slightly more) of water soluble material and 0.1 mg. per kilo of vitamin B_1. On that basis the water soluble material from 1 kilo of manure dissolved in 10 liters of water would contain sufficient vitamin B_1 to exert marked effects upon plant growth (tables 2 and 3). As already mentioned, the lowest effective limit is not known, and it is

[3] We are indebted to David Bonner for carrying out the extraction and fractionation of the manure samples. Details of the work will be published in another connection.

possible that still more dilute solutions of vitamin B_1 may suffice for green plants. A nutrient solution containing 1 mg. of vitamin in 5000 liters exerts a maximum effect in supporting the growth of excised pea roots; one ten times more dilute still possesses a detectable influence (3). Purified extracts of manure (1 mg. of the absolute alcohol fraction of steer manure, representing 200 mg. of air-dry manure, per liter of nutrient medium) were also found to replace pure vitamin B_1 in supporting the growth of isolated pea roots in optimum nutrient solution.

Vitamin B_1 may reach the soil from still other sources. Plant residues contain considerable amounts, for example, and a portion may be returned to the soil after the death of the plant. Soil microorganisms are another possible source. *Azotobacter* cultured on purely synthetic medium is capable of synthesizing considerable amounts of vitamin B_1, and hence is to be regarded as "autotrophic" for this growth factor.

The vitamin B_1 economy of the soil can hardly be dealt with at this time, since it is undoubtedly a complex balance, increased on the one hand by organisms such as *Azotobacter* which produce vitamin B_1 and decreased on the other hand by the vitamin B_1 heterotrophic microorganisms and by some roots.

Discussion

That the growth of many green plants may be limited by the amount of available vitamin B_1 would be expected upon the basis of other experiments of a different nature. It has been shown that for the production of roots on cuttings, auxin, vitamin B_1, and other factors as well are necessary (**17, 18, 19**). Auxin serves only for the initiation of root primordia upon the cutting; for the development of these primordia into roots vitamin B_1 is essential, and root development may be strictly limited by the available amount of this substance. *Camellia* is one example of such a plant. Leafy cuttings of *Camellia* in the experience of this laboratory produce luxuriant roots only if vitamin B_1 is given after roots have been initiated by auxin treatment (**17**). *Camellia* is apparently unable to supply from its leaves sufficient vitamin B_1 for the abundant production of roots on cuttings, and the entire rooted plants respond with increased growth

to the application of vitamin B_1 (table 3). It would seem logical to conclude that *Camellia* normally has available only limited supplies of the vitamin. The leafy cuttings of plants capable of developing visible roots with auxin treatment alone (or even without such treatment) apparently already contain this vitamin, or are capable of manufacturing it in adequate amounts.

The fact that many plants benefit from an external supply of vitamin B_1 by no means indicates that this substance is not a plant hormone. In an analysis of the response of plants to vitamin C, it has been shown (**5**) that those varieties which respond favorably to additions of the substance are those whose capacity for synthesis of it is limited. Plants which synthesize large amounts of vitamin C gave no response to further additions. Vitamin C, however, is certainly to be regarded as a hormone for all plants. The different responses of different plants to vitamin B_1 have not yet been analyzed in a similar manner. It seems safe to predict, however, that many plants produce abundant supplies of this substance and are limited in growth by deficiency of it only under exceptional conditions. On the contrary, other plants almost as certainly produce smaller amounts of vitamin B_1 and their growth is limited by the available supply of it.

It is not desired to overemphasize the role of vitamin B_1 as an "organic fertilizer." The part which specific organic chemicals, special growth factors, present in fertilizers and in the soil may play in plant development has been much overlooked in the recent past. It is hoped that this specific example of such an organic "micronutrient" may serve to induce further investigation in this direction. Not only vitamin B_1, but a host of other specific growth substances—for example auxin, biotin, ascorbic acid, oestrogenic substances, etc. (**2**) —may benefit the growth of green plants under some conditions. The long disparaged "auximones" of BOTTOMLEY (**7, 8**) seem actually to rest upon a sounder basis than has been admitted in the past.

Summary

1. The vitamin B_1 content of pea plants kept in the dark does not increase, whereas in the light this content of the leaf rises rapidly. The root tips of plants whose leaves are in the light contain more

vitamin B_1 than do those of plants kept in the dark. This signifies that vitamin B_1 is produced in leaves in the light and thence transported to the growing root tip.

2. It has been shown that when vitamin B_1 is supplied in small amounts to the roots of plants grown in the dark, both shoot growth and root growth are increased.

3. Numerous species of plants have been grown in nutrient sand cultures in the greenhouse; that is, in the light. Addition of vitamin B_1 (1, 0.1, and 0.01 mg. per liter of nutrient solution) was found to increase the shoot growth of species of plants which are normally slow growing. No additional growth response to added vitamin B_1 was obtained under similar conditions when fast growing annual plants were used.

4. Organic manure contains appreciable amounts of vitamin B_1. The beneficial effects of manure upon plant development may be owing in part to its content of vitamin B_1. The vitamin B_1 content of soils may be expected to be derived also from plant debris and from soil microflora.

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PROTOPLASMIC STRUCTURE IN SPIROGYRA. III. EFFECTS OF ANESTHETICS ON PROTOPLASMIC ELASTICITY[1]

HENRY T. NORTHEN

(WITH ONE FIGURE)

Anesthetics usually affect the rates of many biological processes. For example, at certain concentrations anesthetics cause an initial increase in the rate of respiration which may or may not be followed by a decrease, depending upon the concentration (2). HARVEY (1) suggests that the anesthetics may permit the mixing of certain enzymes and their substrates. He implies that the anesthetics may alter the structure of protoplasm.

NORTHEN (3) found that the rate with which the chloroplasts in cells of *Spirogyra* moved in response to different centrifugal accelerations followed the general equation:

$$V = k(c - c_0)$$

in which V is the velocity of chloroplastic movement, k a constant, c the centrifugal acceleration used, and c_0 the initial starting acceleration at which or below which the chloroplasts will not move regardless of the time the centrifugal acceleration is allowed to act. Because the rate of chloroplastic movement was governed by that equation, it was believed that the protoplasm in cells of *Spirogyra* was an elastic fluid built up of intermeshed linear molecules or micelles.

Stimuli which affect the rate of protoplasmic processes may conceivably alter the structure of protoplasm and the alterations so caused may be detected by changes in the value of c_0, the initial centrifugal starting acceleration. NORTHEN and NORTHEN (4) have demonstrated that the value of c_0 is less than normal in groups of cells adjacent to mechanically killed or heat killed cells. The data

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 164.

in this paper indicate that anesthetics also cause an initial decrease in the value of c_0.

Filaments of *Spirogyra* which had been immersed for different periods of time in several concentrations of various anesthetics were centrifuged, still in the proper anesthetic, with accelerations which were less than the average value of c_0 for untreated filaments. For each experiment about 200 filaments were used. The data were obtained by determining the percentages of filaments in the cells of which the chloroplasts had been displaced (chloroplasts moved to the end of the cell).

TABLE 1

EFFECT OF ETHYL BUTYRATE ON VALUE OF c_0 IN EQUATION $V=k(c-c_0)$

MINUTES IMMERSED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED WHEN IMMERSED IN CONCENTRATIONS OF ETHYL BUTYRATE			
	0%	0.12%	0.25%	0.50%
2	12	16	34	67
8	14	13	11	17
16	10	.....	15	4
30	21	17	26	29

The experiments with ethyl butyrate are summarized in table 1. The filaments were centrifuged with an acceleration of 75.6× gravity for five minutes.

In table 1 it will be noted that when filaments were immersed for two minutes in 0.50 per cent ethyl butyrate and then centrifuged in ethyl butyrate, the chloroplasts were displaced in cells of 67 per cent of the filaments and not displaced in any cells of 33 per cent of the filaments, whereas in the controls the chloroplasts were displaced in cells of only 12 per cent of the filaments. In general it is apparent that the initial effect of 0.25 and 0.50 per cent ethyl butyrate was to decrease the value of c_0, which is interpreted as a decrease in elasticity. Following the initial decrease, c_0 returned to the normal value. Unless the difference between the control (those in 0 per cent) and the anesthetized filaments in this or subsequent experiments was at least 10 per cent, the difference was not considered significant.

Results similar to those recorded in table 1 were obtained when concentrations of 1, 2, and 4 per cent ethyl acetate and when concentrations of 0.125, 0.25, and 0.50 per cent chloral hydrate were used. Again the greatest effects were obtained with the highest concentrations, 4 per cent ethyl acetate and 0.50 per cent chloral hydrate.

TABLE 2

EFFECT OF CHLOROFORM ON VALUE OF c_0

MINUTES IMMERSED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED WHEN IMMERSED IN CONCENTRATIONS OF CHLOROFORM			
	0%	0.062%	0.125%	0.25%
2	1	0	13	100
4	0	1	4	76
8	1	0	2	88
12	5	3	60	86
20	14	15	11	41

TABLE 3

EFFECT OF ETHER ON VALUE OF c_0

MINUTES IMMERSED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED WHEN IMMERSED IN CONCENTRATIONS OF ETHER			
	0%	1%	2%	4%
1	16	.....	16	30
4	14	4	4	25
6	7	8	7	8
26	17	15	19	20

The data for the experiments with chloroform are recorded in table 2. The filaments were centrifuged for five minutes with an acceleration of 75.6× gravity.

The highest two concentrations of chloroform caused an initial decrease in the value of c_0. With the highest concentration the value of c_0 was less than normal after twenty minutes' immersion.

The data for the experiments with ether are recorded in table 3. The filaments were centrifuged with an acceleration of 75.6× gravity for five minutes.

When the filaments were immersed in 4 per cent ether for one minute prior to centrifugation, c_0 was slightly less than normal. With longer immersions it returned to approximately the normal value.

The data for the experiments with ethyl, n propyl, n butyl, and n amyl alcohols are recorded in table 4.

It will be noted (table 4) that in untreated filaments the average value of c_0 varies from day to day. At the time the experiments with

TABLE 4

EFFECTS OF ETHYL, n PROPYL, n BUTYL, AND n AMYL ALCOHOLS ON VALUE OF c_0

Minutes immersed	Centrifugal acceleration (×gravity)	Minutes centrifuged	Percentages of filaments in which chloroplasts were displaced when immersed in				
			Water	Ethyl	Propyl	Butyl	Amyl
				14.5%	6.2%	2.55%	1%
3	75.6	5	5	84	78	85	90
8	75.6	5	26	15	56	52	19
16	108.8	4	94	41	92	92	96
40	108.8	4	97	0	2	83	83
				7.2%	3.1%	1.28%	0.5%
3	42.5	6	5	54	91	90	24
8	42.5	6	4	8	40	71	17
16	42.5	6	2	0	5	65	.....
40	108.8	4	90	51	93	93	96

the higher concentrations were performed c_0 was equal to or less than 75.6 × gravity for at least 74 per cent of the filaments, whereas when the experiments with the lower concentrations were performed c_0 was equal to or less than 42.5 × gravity for about 95 per cent of the filaments.

The alcohols in the concentrations used initially decreased the value of c_0, thus indicating a decrease in elasticity and a likely change in the structure of the protoplasm. Following the initial decrease in protoplasmic elasticity, the elasticity became greater than

normal, as evidenced by an increase in the value of c_0, when 14.5 and 7.2 per cent ethyl alcohol, 6.2 per cent propyl alcohol, 2.55 per cent butyl alcohol, and 1 per cent amyl alcohol were used. However, when the lower concentrations of propyl, butyl, and amyl alcohols were used the elasticity returned to approximately the normal value following forty minutes' immersion.

The following examples illustrate why the chloroplasts are displaced in a greater percentage of filaments which have been immersed for a short time in anesthetics than in those which have not been immersed in anesthetics. The data obtained when 14.5 per cent ethyl alcohol was allowed to act for three minutes will be used as an example. Assume for convenience that k equals 1 in the equation $V = k(c - c_0)$. The velocity of chloroplastic movement was zero for between 74 and 95 per cent (control was quite variable) of the untreated filaments. Hence for about 74 per cent of the filaments the normal value of c_0 was equal to or greater than 75.6 × gravity but less than 108.8 × gravity for about 95 per cent of the filaments (note percentage displacement when acceleration of 108.8 × gravity was allowed to act on filaments in water). Hence for about 74 per cent of the filaments:

$$V = 1(75.6 - 75.6) = 0$$

or:

$$V = 1(75.6 - >75.6) = <0.$$

A velocity less than zero is impossible. Following three minutes' immersion in 14.5 per cent ethyl alcohol, the average value of c_0 had been decreased. Hence for 84 per cent of the filaments:

$$V = 1(75.6 - <75.6) = >0.$$

The chloroplasts will move in 84 per cent of the filaments.

It is believed that stimuli which alter the value of c_0 cause changes in the structure of *Spirogyra* protoplasm. The nature of the prob-

able changes is not known. It may be that the orientation of the long interlaced molecules or micelles is changed or that the molecules or micelles alter their shape. The hypothetical diagrams shown in figure 1 represent one of several possible alterations in structure following short immersions in some anesthetics of the proper concentration. Diagram *a* represents normal protoplasm with a ball suspended in it. The ball will move only when an initial shearing stress, c_0, is exceeded. Diagram *b* represents protoplasm after two

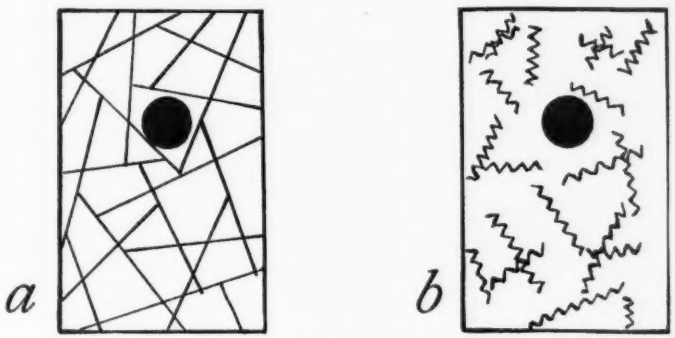

FIG. 1

minutes' immersion in one of the anesthetics used. The shearing stress necessary to cause any movement of the ball has been decreased.

Summary

1. Filaments of *Spirogyra* which had been immersed for different periods of time in several concentrations of various anesthetics were centrifuged with accelerations which usually caused no displacement of the chloroplasts in most cells of non-anesthetized filaments.

2. The initial effect of certain concentrations of the anesthetics was to decrease the value of c_0 in the equation, $V = k(c - c_0)$, which approximately governs the rate with which the chloroplasts in cells of *Spirogyra* move in response to centrifugal accelerations. A decrease in c_0 was interpreted as a decrease in protoplasmic elasticity.

3. With longer immersions c_0 returned to approximately the normal value except in the case of the alcohols, in which instances c_0 became greater than normal (elasticity increased).

I am deeply grateful to my wife and to Mr. RALPH AMES for aid in obtaining data.

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EFFECTIVENESS OF PHOTOPERIODIC TREATMENTS OF PLANTS OF DIFFERENT AGE

H. A. BORTHWICK[1] AND M. W. PARKER[2]

It has been reported by CAJLACHJAN (**2**) that short day treatments applied to millet were most effective when the plants were one and five weeks old, and that it was at these periods that millet made its most rapid growth.

In the present investigation the Biloxi soy bean, which is very responsive photoperiodically, was used. Critical morphological examinations were made of the fresh material before and after short day treatments. It is unfortunate that many investigators in this field have failed to examine their material critically; thus the results are difficult to interpret. Six plantings of about 150 plants each were made in the greenhouse at weekly intervals from January 21 until February 25, 1938. All plants were grown at the intensity and duration of natural light then prevailing, supplemented by approximately 50 foot candles of Mazda light from sundown until midnight.

From among the 150 plants of each planting, 72 of the most uniform in size and appearance were again selected. These were then divided into six lots of twelve plants each. Beginning one week after planting, one lot was subjected to an induction treatment of four 8-hour days and then returned to a 16-hour day to continue development. At the end of the second week another lot was similarly treated, and this procedure was continued until all six lots had been similarly disposed of. The last lot received its induction treatment six weeks after planting. Except for the induction periods of four 8-hour days, none of the plants was subjected to less than a 16-hour day.

Two weeks after the beginning of the induction treatment, six plants from each lot were dissected and examined in detail to de-

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termine the number and location of flower buds. Three weeks later the remaining plants of each lot were similarly examined.

It has previously been shown (1) that the first response of the Biloxi soy bean to a short photoperiod occurred in the axil of the leaf that was approximately third from the tip of the main axis when the treatment was started. Similar response also occurred simultaneously approximately three nodes from the tips of certain axillary buds. In the experiment here reported reference is made only to flower primordia initiated on the main axis.

If treatment with short photoperiod is continued for many days, flower primordia are eventually initiated at all the nodes above the point where the first flower buds were found. With treatments of only four 8-hour days, however, flower primordia may arise at a few nodes; but under the influence of 16-hour days following the induction period there is an increasing tendency toward differentiation of shoots instead of flower primordia at the newly forming nodes above. The number of nodes at which flower primordia are differentiated affords a measure of the effectiveness of identical treatments given to plants of different ages.

The number of plants forming flower primordia in response to identical photoperiodic treatments increased with the age of the plant (table 1). No plants responded when treated one week after planting, and only nine out of seventy-two showed any response when two weeks old. Most of the 3- and 4-weeks-old plants and all of the 5- and 6-weeks-old ones formed flower primordia under similar treatment.

The treatments at one date constitute an exception to this relationship. The 3-weeks-old lot of planting series 5 and the 4-weeks-old lot of planting series 4, which were given short day treatment beginning March 11, failed to make visible response. All the 5-weeks-old plants of lot 3 and the 6-weeks-old ones of lot 2 treated at the same time, however, produced flower primordia. The plants of all these lots were kept together and consequently received exactly the same treatment. The difference in their response is unexplained but is apparently correlated with their age or size. At the time this particular treatment was given, the night temperature of the green-

house was about 5° higher than was true with the other treatments, and the light intensity was very low because of stormy weather.

TABLE 1

NUMBER OF PLANTS IN EACH TREATMENT SHOWING FLOWER BUD FORMATION IN RESPONSE TO INDUCTION PERIOD

Planting series	Age at time induction treatments were started (weeks)					
	1	2	3	4	5	6
1	0	3	10	10	12	12
2	0	2	11	12	12	12
3	0	0	12	12	12	12
4	0	3	11	0	12	12
5	0	1	0	12	12	12
6	0	0	12	12	12	12
Total	0	9	56	58	72	72

TABLE 2

Age at start of induction period (weeks)	Total number of nodes per plant at which flower primordia were initiated		Total nodes present per plant at start of induction period	Location of flower primordia (nodes from base of plant)			
				First flower primordia formed		Last flower primordia formed	
	At end of 2 weeks*	At end of 5 weeks		At end of 2 weeks	At end of 5 weeks	At end of 2 weeks	At end of 5 weeks
1	0	0	.....	.....	.....	.....	.....
2	1.1	1.0	.....	6.7	7.0	6.8	7.0
3	1.8	1.7	10	8.8	8.6	9.6	9.3
4	2.3	2.1	12.5	10.5	10.5	11.8	11.6
5	2.9	2.8	14.9	11.8	12.2	13.7	14.0
6	4.1	4.4	16.6	13.4	13.6	16.5	17.0

* Time measured from beginning of induction period.

Flower primordia were produced at more nodes per plant on the older than on the younger plants (table 2). Evidently the stimulus of four 8-hour days resulting in differentiation of flower buds has a

greater effect if it is received after the plants have attained greater age and size. The development of all flower primordia was apparently well advanced within two weeks after the start of treatment, for there was no significant difference in the number found then and three weeks later (table 2).

Comparison of the figures in table 2 showing the position of the uppermost flower primordia with those showing the total number of nodes present at the beginning of treatment indicates that flower primordia were initiated in the axils of leaves already present at the time the treatments were begun. One possible exception is in the 6-weeks-old lots. Although this suggests that there is no "carry-over" effect of an induction period to structures differentiated subsequent to it, later experiments made during the summer show that there is such an effect. In these later experiments flower primordia have been found at positions several nodes above the node which was the uppermost one when the induction period was terminated.

The results of this experiment show that the effectiveness of a photoperiodic treatment consisting of four 8-hour days increases with the age of the plant to which it is applied until the plants are at least six weeks old. It would be desirable to investigate the responses of still older plants. The complete failure on one occasion of 3- and 4-weeks-old plants to respond to the same treatment that resulted in flower bud initiation in 5- and 6-weeks-old plants indicates that other environmental factors may influence the photoperiodic response. Data which will be presented at another time show that the intensity of illumination during treatment exercises a controlling influence on the response. Temperature, humidity, and other factors also play a part, and under conditions of very brief photoperiodic treatment these factors other than the length of the period of illumination may become limiting.

The reason for differences in effectiveness of photoperiodic treatments on plants of different ages is probably associated with differences in leaf area. CAJLACHJAN (3) has called attention to the fact that plants respond photoperiodically as soon as the first green leaf appears and not before. The data here presented are in agreement with this observation. Other data, which will be published later,

show definitely that the response of the Biloxi soy bean to photoperiodic treatment is mainly an expression of the stimuli to which the leaves have been subjected.

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A MICRO-KJELDAHL METHOD INCLUDING NITRATES

RUFUS H. MOORE

To those who find it necessary to use a micro-method for the determination of total nitrogen in the presence of nitrates, the following procedure is offered.

This procedure is an adaptation of the reduced iron macro-method of PUCHER, LEAVENWORTH, and VICKERY (3) to the micro-method described by PREGL (2). As the former requires 35 cc. of concentrated H_2SO_4 and the latter only 1 cc., the adaptation consists essentially of reducing all reagents employed, up to the point of adding the catalyst, to 1/35 of that recommended by the authors of the reduced iron method.

The complete Eimer and Amend micro-Kjeldahl apparatus was used for the tests reported. In the modified procedure, from 10 to 50 mg. of powdered sample were used. To the sample in the digestion flask 1 cc. water, 0.29 cc. (1 + 1) H_2SO_4, and 0.086 ($\pm$0.0086) gm. iron powder were added. The flask was shaken ten minutes and cooled slightly under tap water (if necessary) to prevent excessive frothing. The flask was then heated five minutes over a very low flame and allowed to cool. After adding 0.86 cc. concentrated H_2SO_4, a knife point of a 1:3 mixture of K_2SO_4 and $CuSO_4$, and two small angular quartz pebbles, digestion over a medium flame was allowed to continue until fumes appeared. Nitrogen-free Superoxyl was added until the solution remained blue. After heating over a high flame for one hour, the flask was allowed to cool slightly and 1 cc. of water added. The sample was distilled as described by PREGL, a methyl red-methylene blue indicator having been used in titration.

Powdered samples were weighed on small pieces of cigarette paper, kept between paired watch glasses during weighing to prevent excessive absorption of moisture. The paper was folded over the sample in such a way that it would open readily when dropped into the bulb of the digestion flask and the latter shaken, thus dispersing the sample for rapid solution in the acid. The cigarette paper used

was free from nitrogen, digested readily, was sufficiently fine-pored to retain the sample, and flexible enough to permit easy handling.

TABLE 1

SUMMARY OF PRECISION TESTS OF REDUCED IRON MICRO-KJELDAHL METHOD

Material tested	No. of trials	0.01 N equivalent of sample (cc.)	Nitrogen found (mg.)
Blank	6	0.351±0.0056	0.049
$NaNO_3$	5	1.079±0.0096	0.151
Witte's peptone	3	2.524±0.0067	0.359
$NaNO_3$ and Witte's peptone	3	3.627±0.0044	0.514

TABLE 2

TOTAL NITROGEN IN PEANUT TISSUES AS DETERMINED BY REDUCED IRON MICRO-KJELDAHL METHOD

Plant fraction	Weight of sample (mg.)	Total nitrogen (%)	Deviation from mean (%)
Primary root and hypocotyl	24.78	0.690	±1.71
	21.32	0.714	
Green gynophores	27.44	2.266	±1.52
	18.31	2.336	
Mature gynophores	26.23	1.980	±0.69
	25.57	1.953	
Mature gynophores	26.93	1.382	±0.50
	23.17	1.396	
Immature fruits	12.59	3.907	±1.24
	10.75	4.005	
Immature fruits	11.31	5.362	±0.00
	15.26	5.362	
Mature seeds	45.88	2.945	±0.62
	52.64	2.982	

For determination of nitrogen in alcoholic extracts 1 cc. aliquots should be used. Iron powder was measured in a small aluminum spoon.

The precision of the foregoing adaptation was tested on a solution of $NaNO_3$, Witte's peptone dissolved in 2 per cent H_2SO_4, and a mixture of these two. As each sample of $NaNO_3$ was calculated to contain 0.1502 mg. of nitrogen and each of the peptone aliquots determined to contain 0.3592 mg. of nitrogen, 29.5 per cent of the nitrogen in the combined sample was nitrate nitrogen. Sufficient distilled water was added in each case to bring the volume of the sample to 1 cc. before reagents were added. Results are condensed in table 1. The average recovery of nitrogen from the $NaNO_3$ samples was calculated as 100.64 per cent, and from the combined samples of $NaNO_3$ and Witte's peptone as 100.85 per cent. More extensive tests of the precision of this method are to be desired.

The method was used on certain ovendried fractions of the peanut plant, which provided too small an amount of tissue for macroanalysis. Results are given in table 2. This method was also used by JONES (1) to determine total nitrogen in the presence of nitrates in wheat seedlings.

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CURRENT LITERATURE

General Plant Physiology. By E. C. BARTON WRIGHT. London: Williams and Norgate Ltd., 1938. Pp. 539.

A new textbook of plant physiology from an English plant physiologist will be read with much interest by American students of the subject. The author is well known by reason of his Recent Advances in Plant Physiology, which appeared in two editions, 1930 and 1933. The new work is elementary, intended for first and second year students in colleges and universities. The material is presented in three parts: general physiology of the cell; metabolism; and growth, reproduction, and irritability.

The first part includes chapters on the scope of physiology, colloids and protoplasm, osmotic pressure and the water relations of the plant, permeability, transpiration, and the ascent of sap. The metabolism section offers chapters on catalysis and enzymes, photosynthesis, the fats, nitrogen metabolism, ash, the transport of solutes, and respiration. The final section includes germination, growth, reproduction, and irritability and plant movements. A brief appendix discusses pH, following which there is a bibliography of 87 titles, mainly texts or monographs, and author and subject indexes.

Readers should keep in mind the great difficulty confronting anyone who writes a text on a subject which advances as rapidly as plant physiology has during the last fifteen years. Before any text can be published, some of the material is almost certain to be superseded by newer work. The foreword by F. GOWLAND HOPKINS calls attention to the logical arrangement of the presentation, and to the relative independence of the sections, so that individual chapters may be read with satisfaction. English plant physiologists have contributed much to the development of our knowledge, and American students should be given an opportunity to read and appreciate this interesting and very readable text.—C. A. SHULL.

General Physiology. By PHILIP H. MITCHELL. New York: McGraw-Hill Book Co., 1938. Pp. xviii+853. $6.00.

Laboratory Manual of General Physiology. By PHILIP H. MITCHELL and IVON R. TAYLOR. New York: McGraw-Hill Book Co., 1938. Pp. xv+142. $1.50.

The third edition of MITCHELL's General Physiology is now accompanied by a useful laboratory manual, designed to offer choice for a year of laboratory study of physiological problems. The text has been carefully revised, with extensive changes in the chapters dealing with excitation, contraction, transmis-

sion, membrane structure, cell permeability, physiological oxidations, muscle chemistry, vitamins, and hormones. These changes have been dictated by the rapid developments in these fields. New sections deal with organizers or evocators, oxidation-reduction potentials, Liesegang phenomena, and the physiological study of temperature characteristics. The discussion brings to the student a knowledge of the improved techniques which have been developed since the second edition appeared, and the new theories of physiological action are given adequate attention for a work designed for a single course in general physiology. Some physiologists may criticize the choice of material, but it attempts to present some phases of plant physiology while placing the main emphasis on animal physiology.

The laboratory manual follows the order of presentation of the text, and offers a wide choice of materials. The text contains twenty-five chapters, and twenty-one of these are represented in the manual. There are 254 laboratory exercises, many more than can be used in a full year course. Instructors may thus adapt the work to individual laboratories by choice in accordance with equipment, or in accordance with the emphasis desired. The reviewer finds them interesting and valuable. Teachers will surely welcome the addition of the laboratory manual, which should make the use of the text feasible in any institution which possesses good laboratory equipment.—C. A. SHULL.

Cryptogamic Botany: Vol. I. Algae and Fungi. By GILBERT M. SMITH. New York: McGraw-Hill Book Co., 1938. Pp. vi+545. Figs. 299. $4.00.

As stated in the preface, "this book is designed for students who have had an introductory course in botany and who wish to make a more detailed study of plants below the level of the seed plants. It is written from the standpoint that a thorough knowledge of a representative series in each of the major groups is better than scraps of information about a large number." With this purpose in mind, the author has brought together representative examples of the various divisions and classes of the algae and the fungi.

Following chapter I, which discusses the classification of spore-producing plants, some 338 pages are devoted to the algae and 172 to the fungi. The algae are presented under seven divisions: Chlorophyta or grass-green algae, Euglenophyta or euglenoids; Pyrrophyta, which includes cryptomonads and the dinoflagellates; Chrysophyta, which includes the yellow-green algae (Xanthophyceae), the golden-brown algae (Chrysophyceae), and the diatoms; Phaeophyta or brown algae; Cyanophyta or blue-green algae; and the Rhodophyta or red algae. The fungi are presented under two divisions, Myxothallophyta or slime molds and Eumycetae, or true fungi, embracing the classes of Phycomycetes, Ascomycetes, Basidiomycetes, and the Fungi Imperfecti.

The examples used throughout the text have been carefully chosen, and the details of their life cycles have been clearly and, in the main, completely covered.

The illustrations are largely original, are judiciously selected, and exceptionally well executed. Both the author and the publisher are to be commended for their work.

A feature which merits comment is that this is the first attempt which has been made to bring together in one volume a fairly condensed treatment of the principal groups of the algae, and thus make available to English speaking students the essential features of the life histories of the representatives chosen. This is a noteworthy achievement, and would of itself make the book a real contribution.

Some botanists may think that too little space has been given to the fungi, but the reviewer is of the opinion that the author has succeeded in accomplishing his purpose of adequately presenting selected representative forms covering the principal phylogenetic tendencies and the significant types of reproductive structures, including the nuclear phases. The book is suitable for a semester course covering the algae and the fungi, or for two quarter courses devoted to each group separately.—J. M. BEAL.

Cryptogamic Botany: Vol. II. Bryophytes and Pteridophytes. By GILBERT M. SMITH. New York: McGraw-Hill Book Co., 1938. Pp. vii+380. Figs. 224. $3.00.

The author has brought together the essentials of the present day knowledge about bryophytes and pteridophytes in a simple, direct manner that will make it usable both for class work and for reference. While the text is necessarily abbreviated, the bibliographies are cited in such a manner that the study may be greatly expanded when desired. Illustrations are ample and well chosen. In general they are well described and lettered.

The subject matter is consistently arranged according to taxonomic classification. The distinguishing characteristics of each class, order, and family are discussed. In each family the distribution, structure, and life histories of representative genera are described. The first 113 pages are devoted to the Bryophyta, while 253 pages deal with the Pteridophyta, both fossil and living.

In the Bryophyta the Anthocerotae are regarded as a group of equal rank with the Hepaticae and Musci. The Pteridophyta are arranged in four large groups, the Psilopytinae, Lycopodinae, Equisetinae, and Filicinae.

In discussing the origin of the Pteridophyta, the author recognizes the present tendency to derive the group from algal ancestors. He, however, presents a convincing discussion of its possible origin from ancestors of the anthocerotean type. Theories as to the origin of the vascular system and its evolution among Pteridophyta constitute a valuable and interesting part of the introduction to this group.

This book will fill a real need for a textbook on the archegoniates.—ELDA R. WALKER.

Manual of Pteridology. Edited by FR. VERDOORN. The Hague: Martinus Nijhoff, 1938. Pp. xv+640. Illustrated. 24 guilders.

This is a collection of essays by twenty specialists on various subjects dealing with pteridophytes. It is intended primarily to broaden the background of taxonomists but it will be of equal value to those interested in ferns from other viewpoints. There is scarcely a phase of information in connection with the group that is not reviewed.

The foreword by F. O. BOWER is followed by chapters on morphology, anatomy, experimental morphology, cytology, genetics, growth and tropisms, chemistry, ecology, geography, pathology, symbiotism both with algae and with mycorhizal fungi, gall formations, and phylogeny. About half of the chapters are in English; the remainder in German. By means of footnotes and bibliographies much literature is made available.

The volume is a most valuable compendium of information along all lines dealing with pteridophytes, both living and fossil.—ELDA R. WALKER.